

First Edition . . . 1934
Spanish Edition . . . 1936
Second Edition . . . 1940

BY THE SAME AUTHOR
NUTRITIONAL FACTORS IN DISEASE

AN INTRODUCTION TO BIOCHEMISTRY

WILLIAM ROBERT FEARON

M.A., Sc.D., M.B., F.I.C.

FELLOW OF TRINITY COLLEGE, DUBLIN

MEMBER OF THE ROYAL IRISH ACADEMY

SECOND EDITION



LONDON
WILLIAM HEINEMANN
(MEDICAL BOOKS) LTD.
1940

TO
SIR FREDERICK GOWLAND HOPKINS

*What is but now compleat and done
Was long before resolved on.*

THE CHYMICAL MARRIAGE OF CHRISTIAN ROSENCREUTZ

PREFACE TO THE SECOND EDITION

SINCE the preparation of this edition has required the re-writing of about three-quarters of the entire book, it hardly seems necessary to specify more than the principal alterations. Solutions and Colloidal Systems, Steroids, Pigments (Pyrrole Derivatives, Carotinoids, Flavins), Tissue Respiration, and Internal Environment are now made the subject of special chapters. New analytical methods include the citrulline reaction for proteins and the diffusion test for acetone. Owing to the kindness of Dr. E. J. King I have been able to reprint three of his micro-methods for blood analysis.

For information and for copies of scientific papers I am indebted to many correspondents, including Professor A. Hunter, Professor E. C. Dodds and Dr. R. A. McCance. I am also indebted to Professor A. P. Vinogradov, who has provided me with the Transactions of the U.S.S.R. Academy of Sciences necessary for my chapter on intrinsic Biological Elements; to Alexander Lieven, for his translations from the original Russian monographs, and for the preparation of the Index to my book; to Sir Joseph Barcroft, F.R.S.; and to my colleagues, Professor D. S. Torrens and Dr. James Bell.

Figs. 1 and 2 are reproduced, by kind permission, from Professor Niels Bjerrum's *Inorganic Chemistry* (translated by R. P. Bell), and Fig. 3 is from Dr. Sherwood Taylor's *Organic Chemistry*, both of which works will be found very helpful in the approach to biochemistry.

Finally, my thanks are due to my publishers for their unfailing assistance during an unforgettable time.

W. R. F.

DUBLIN, 1940

PREFACE TO THE FIRST EDITION

I HAVE sought to approach the living organism, and to lead such as may be disposed to follow me, along the less worn path of *inorganic biochemistry*. This mode of approach, which demands a consideration of the ultimate ingredients of our dusty framework, has been somewhat neglected, partly perhaps because of the dryness that is associated with the subject-matter, and partly because the facts are widely scattered, tedious to collect, and often hard to reconcile. Still, the increasing interest in the micro-essential constituents of tissues reminds us of the fundamental importance of the chemical elements associated with life.

In the subsequent excursions I have taken various short cuts, chiefly by omitting much in the way of organic chemistry. I have also, with little regret, avoided some regions of tissue chemistry, especially blood, muscle, and nerve, as it is difficult to survey them adequately without physiological and histological assistance outside the scope of this venture. The time thus saved has been devoted to the practical description of illustrative tests, including several unpublished observations.

The distracting question of references in an elementary text-book I have tried to answer by including lists of review articles, many of which are heavily documented. Additional statements and dated references will, I hope, be traceable by means of the invaluable *Annual Report Series* of the Chemical Society, the *Annual Review of Biochemistry*, *Biological Abstracts*, *Physiological Abstracts*, and the *Abstracts* of the British and the American Chemical Societies.

W. R. F.

TRINITY COLLEGE
DUBLIN

“ We carry with us the wonders we seek
without us : there is all Africa and her
prodigies in us.”

THOMAS BROWNE

TABLE OF CONTENTS

CHAPTER

PAGE

PART I. ELEMENTS AND INORGANIC COMPOUNDS

I. THE SUBJECT MATTER OF BIOCHEMISTRY . . .	1
II. BIOLOGICAL ELEMENTS	7
III. INORGANIC COMPOUNDS	45
IV. SOLUTIONS AND COLLOIDAL SYSTEMS	62

PART II. ORGANIC COMPOUNDS

V. CLASSIFICATION AND CHARACTERISTICS . . .	72
VI. CARBOHYDRATES.	76
VII. REACTIONS OF CARBOHYDRATES	102
VIII. PROTEINS	125
IX. AMINO ACIDS AND PROTEIN STRUCTURE . . .	135
X. LIPIDES	158
XI. STEROIDS	172
XII. PIGMENTS : PYRROLE DERIVATIVES, CAROTINOIDS, FLAVINS	184
XIII. CATALYSTS.	208
XIV. NUTRIENTS	234
XV. ALIMENTARY DIGESTION	266
XVI. INTERMEDIATE METABOLISM CARBOHYDRATES	282
XVII. INTERMEDIATE METABOLISM PROTEINS . . .	303
XVIII. INTERMEDIATE METABOLISM : LIPIDES . . .	316
XIX. TISSUE RESPIRATION	325

CHAPTER	PAGE
XX. PURINES AND PYRIMIDINES.	344
XXI. NITROGENOUS BASES	356
XXII. UREA	376
XXIII. EXCRETION	390
XXIV. HORMONES.	412
XXV. THE INTERNAL ENVIRONMENT	449
APPENDIX	459
INDEX	465

AN INTRODUCTION TO BIOCHEMISTRY

PART I

INORGANIC BIOCHEMISTRY

CHAPTER I

THE SUBJECT-MATTER OF BIOCHEMISTRY

“Definitions, formulæ (some would add, creeds) have their use in any society in that they restrain the ordinary unintellectual man from making himself a public nuisance with his private opinions.”

ARTHUR QUILLER-COUCH.

Biochemistry is the study and the interpretation of the chemical changes associated with life. Life is indefinable, but is recognisable by its manifestation in material organisms composed of two or three dozen relatively common chemical elements. These organisms are autonomous systems displaying the following properties: adaptiveness, growth, repair, reproductiveness. From the biochemical standpoint, *an organism is a system that maintains its identity by means of self-controlled physical and chemical changes.*

Physics and Chemistry.—Matter may undergo three kinds of transformation: (1) molecular rearrangement, as in the conversion of water into ice; (2) molecular decomposition and atomic rearrangement, as in the electrolysis of water into hydrogen and oxygen; (3) nuclear decomposition, as in the transformation of lithium into helium. A physical change merely involves molecular rearrangement; a chemical change involves atomic rearrangement with the production of new molecules; a sub-atomic change involves rearrangement in electron systems, and production of new atoms. Biochemistry is concerned primarily with the composition of plant and animal structures and products, and the atomic rearrangements taking place in plants and animals. Many of these chemical changes are accompanied by important physical events which are often included in the study of biochemistry, although, strictly, they belong to the related sciences of biophysics and physiology. Sub-

atomic changes have not yet been shown to be of significance in living organisms.

Development of Biochemistry.—A distinction between the chemistry of living and non-living substances is implicit in the writings of the alchemists, one of whom, as Dr. Needham has pointed out, gave biochemistry its charter when he wrote :—

“The Body is a conglomeration of chemical matters; when these are deranged, illness results, and nought but chemical medicines may cure the same.”

THEOPHRASTUS PARACELSUS, 1527.

In 1675, Nicholas Léméry, the author of the first rational textbook on chemistry, classified the science as *animal*, *vegetable*, and *mineral*. Lavoisier, about 1770, observed that animal and vegetable material differed from minerals in being very rich in carbon, hydrogen, and oxygen. Subsequently, he detected nitrogen and phosphorus in animal matter, and concluded that in this respect it differed from vegetable matter. Later work showed that this conclusion was false; all plants contain nitrogen and phosphorus, although these elements may be absent from some vegetable products. As a result, animal and plant chemistry were grouped together under the name of *organic chemistry*, in distinction from *inorganic* or *mineral chemistry*.

Early in the nineteenth century it was assumed that a fundamental difference existed between these two classes, organic compounds being the exclusive products of vital activity. Then, in 1828, Wöhler, a young German chemist, accidentally obtained urea from ammonium cyanate, by the action of heat. Urea, the chief nitrogenous solute of mammalian urine, is a typical organic compound; indeed, from the point of view of a vegetable it would be regarded as the chief useful product of the animal kingdom. Wöhler's startling discovery altered the outlook of chemistry. The synthesis of other vital products gradually followed, and by the end of the century the term *organic chemistry* had lost its original vitalistic implications, and was applied to all the combustible compounds of carbon, irrespective of their natural or artificial origin. In the meantime, the chemistry of animal and plant materials had been pursued under such titles as: medical chemistry, agricultural chemistry, pathological chemistry, and chemical physiology, until the term *biological chemistry* (abbreviated to the hybrid, *biochemistry*) was introduced to include all applications of chemistry to the study of life and its products.

Vitalism, Mechanism, and Organicism.—To a vitalist, the changes in a living organism are not completely explicable in terms of

physics and chemistry. To the mechanist, life is a manifestation of higher material properties, which, given suitable conditions, appear along with the other products of chemical reactions, and are equally devoid of purpose. To the organicist, or neo-vitalist, there is neither life-substance nor life-force, but, instead, there is a specific life-structure within which various changes take place according to the laws of physics and chemistry. These metaphysical considerations are outside the scope of most workers, who well may agree with Whitehead that :—

“ The mode of approach to the problem, so far as science is concerned, is merely to ask if molecules exhibit in living bodies properties which are not to be observed amid inorganic surroundings.”

To a disembodied and detached observer, a living organism is only a form in which carbon is collected, stored and oxidised. Why this should occur under these restricted conditions, or whether the organism has any cosmic function other than to delay the dissipation of solar energy, is a problem beyond the horizon of the biochemist, whose task is to find what changes occur and how they are brought about. The existence of life is accepted as a self-evident fact in the hope that man's experience of it will eventually enable him to understand its meaning.

The Four-Dimensional Organism.—In the early period of organic chemistry it was possible to represent compounds by two-dimensional formulæ on paper, but the discovery of optical isomerism made it necessary to employ three-dimensional perspective formulæ to show the distinction between related compounds. The living organism is more complex than a tri-dimensional solid ; it is a structure undergoing changes in time as well as in space, and its composition and pattern at a given moment must be considered in relation to its previous and future history. This concept was present in the mind of Bergson when he wrote : “ Doubtless we think with only a small part of our past, but it is with our entire past that we desire, will and act.” Knowledge of the earlier states of an organism enables an observer to predict the immediate future states, and for this reason serial observations are becoming more widely used. When possible, biochemical data, such as gastric acidity or blood sugar values, are presented in the form of time-concentration curves instead of single analyses.

World-Volume.—Regarded as a four-dimensional system, man is an organism occupying a certain amount of space—which, during most of his life, is about 70 litres—for a certain number of years—usually 65–70. By multiplying these values a new quantity, the *world-volume*, or *stereochrone*, is obtained which expresses the history of the organism. The world-volume of the human subject is about 5,000 litre-years,

starting from the fertilised ovum of 0.004 cu.mm. The composition of an organism at a given instant represents a cross-section of its world-volume at that instant.

Subdivisions.—Biochemistry may be classified according to the material examined and according to the purpose of the examination. The first subdivision includes plant biochemistry, animal biochemistry, human biochemistry, cytological chemistry, tissue chemistry, embryological chemistry, bacterial chemistry, enzyme chemistry, and the like. The second subdivision includes physiological, pathological, clinical, medical, industrial, analytical and theoretical biochemistry. A more or less practical form of the science is now part of medical education, and is pursued partly for the information it gives about the normal working of the human machine, partly for the means it affords of detecting and measuring pathological conditions, and partly for the weapons it offers in the chemical warfare against disease and death.

Literature.—Biochemical research is in rapid progress throughout the civilised world, and the output of work is overwhelming. The main channels of information are scientific periodicals, reviews, textbooks and monographs.

Among the most important publications are: *The Biochemical Journal*, *The Journal of Biological Chemistry*, *Zeitschrift für physiologisches Chemie*, *Biochemisches Zeitschrift*, *Bulletin de la société de chimie biologique*, *Acta Phytochemica*, *Annual Review of Biochemistry*, *Annual Reports of the Chemical Society of Great Britain*, *Biological Abstracts*, *Biological Reviews*, *Nutrition Abstracts and Reviews*, *Chemical Reviews*, *Tabula Biologica*, *Chemical Abstracts* (British and American), *Ergebnisse der Physiologie*, *Symposia on Quantitative Biology*, *Enzymologia*, *Ergebnisse der Enzymforschung*, *Ergebnisse der Vitamin- und Hormonforschung*, *The Journal of General Physiology*, *Monographs on Biochemistry*, *Les Problèmes Biologique*.

Textbooks are dominated by the monumental *Biochemisches Handlexicon* of Abderhalden, and the *Handbuch der Biochemie* of Oppenheimer. In the English language, the most familiar are the books by Bodansky, Cameron, Cole, Gortner, Halliburton, Heilbrunn, Harrow and Sherwin, Hawk and Bergeim, Koch, McClendon, Mathews, Milroy, Morse, Parsons, Peters and Van Slyke, Plimmer, Pryde, Robertson, Sumner, Thorpe, and Williams. A valuable survey of scientific books is *The Literature of Chemistry*, by E. J. Crane and A. M. Patterson.

Course of Study.—The plan most usually adopted in studying animal biochemistry is to start with the general composition of the organism, and the chemical characteristics of the chief organic and

inorganic compounds that enter into its structure. Then, the chemical composition of the tissues and physiological systems is examined, and tissue properties are explained in terms of tissue chemistry. Finally, the composition of the diet is investigated, along with the significance of each constituent, its changes during digestion, and its intermediate metabolism and forms of excretion. Thus, starting with static, analytical biochemistry, the student proceeds to explore the innumerable and interlinked reactions that determine, in part, at least, the behaviour of "the thing called Man."

GENERAL REFERENCES

- BAYLISS, W. M. (1924), "Principles of General Physiology." 4th Ed., London.
- "Perspectives in Biochemistry." Edited by J. Needham and D. R. Green (1937), Cambridge.
- HOPKINS, F. G. (1933), "Some chemical aspects of life," British Association Address. *Nature*, **132**, 381.
- HOPKINS, F. G. (1932), "Some aspects of biochemistry," Purser Memorial Lecture. *Irish Jour. Med. Sci.*, July, 1932.
- HOGBEN, L. (1930), "The Nature of Living Matter," London.
- WOODGER, J. H. (1929), "Biological Principles," London.

ATOMIC WEIGHTS

Atomic number.	Name.	Sym-bol.	Atomic weight.	Atomic number.	Name.	Sym-bol.	Atomic weight.
1	Hydrogen .	H	1.0081	47	Silver .	Ag	107.880
2	Helium .	He	4.003	48	Cadmium .	Cd	112.41
3	Lithium .	Li	6.940	49	Indium .	In	114.76
4	Beryllium .	Be	9.02	50	Tin .	Sn	118.70
5	Boron .	B	10.82	51	Antimony .	Sb	121.76
6	Carbon .	C	12.01	52	Tellurium .	Te	127.61
7	Nitrogen .	N	14.008	53	Iodine .	I	126.92
8	Oxygen .	O	16.0000	54	Xenon .	Xe	131.3
9	Fluorine .	F	19.00	55	Cæsium .	Cs	132.91
10	Neon .	Ne	20.183	56	Barium .	Ba	137.36
11	Sodium .	Na	22.997	57	Lanthanum .	La	138.92
12	Magnesium .	Mg	24.32	58	Cerium .	Ce	140.13
13	Aluminium .	Al	26.97	59	Præseodymium	Pr	140.92
14	Silicon .	Si	28.06	60	Neodymium .	Nd	144.27
15	Phosphorus .	P	31.02	61	Illinium .	Il	—
16	Sulphur .	S	32.06	62	Samarium .	Sm	150.43
17	Chlorine .	Cl	35.457	63	Europium .	Eu	152.0
18	Argon .	A	39.944	64	Gadolinium .	Gd	156.9
19	Potassium .	K	39.096	65	Terbium .	Tb	159.2
20	Calcium .	Ca	40.08	66	Dysprosium .	Dy	162.46
21	Scandium .	Sc	45.10	67	Holmium .	Ho	163.5
22	Titanium .	Ti	47.90	68	Erbium .	Er	167.2
23	Vanadium .	V	50.95	69	Thulium .	Tm	169.4
24	Chromium .	Cr	52.01	70	Ytterbium .	Yb	173.04
25	Manganese .	Mn	54.93	71	Lutecium .	Lu	175.0
26	Iron .	Fe	55.84	72	Hafnium .	Hf	178.6
27	Cobalt .	Co	58.94	73	Tantalum .	Ta	180.88
28	Nickel .	Ni	58.69	74	Tungsten .	W	183.92
29	Copper .	Cu	63.57	75	Rhenium .	Re	186.31
30	Zinc .	Zn	65.38	76	Osmium .	Os	190.2
31	Gallium .	Ga	69.72	77	Iridium .	Ir	193.1
32	Germanium .	Ge	72.60	78	Platinum .	Pt	195.23
33	Arsenic .	As	74.91	79	Gold .	Au	197.2
34	Selenium .	Se	78.96	80	Mercury .	Hg	200.61
35	Bromine .	Br	79.916	81	Thallium .	Tl	204.39
36	Krypton .	Kr	83.7	82	Lead .	Pb	207.21
37	Rubidium .	Rb	85.48	83	Bismuth .	Bi	209.00
38	Strontium .	Sr	87.63	84	Polonium .	Po	—
39	Yttrium .	Y	88.92	85	—	—	—
40	Zirconium .	Zr	91.22	86	Radon .	Rn	222
41	Niobium .	Nb	92.91		(Emanation) .		
	(Columbium) .	(Cb)		87	—	—	—
42	Molybdenum .	Mo	95.95	88	Radium .	Ra	226.05
43	Masurium .	Ma	—	89	Actinium .	Ac	—
44	Ruthenium .	Ru	101.7	90	Thorium .	Th	232.12
45	Rhodium .	Rh	102.91	91	Protoactinium	Pa	231
46	Palladium .	Pd	106.7	92	Uranium .	U	238.07

CHAPTER II

BIOLOGICAL ELEMENTS

“This was the heavenly hiding-place
Wherein the spirit laughed a day.
All its proud ivories and fires
Shrunk to a shovelful of clay.”

GEORGE RUSSELL.

BIOLOGICAL elements vary greatly in importance and in distribution. Some are present in all forms of life, others have been detected only in a few species. Some are present in large amounts, others occur as traces of doubtful significance.

CLASSIFICATION OF THE BIOLOGICAL ELEMENTS

Of the ninety-two elements believed to constitute the universe, at least sixty have been found by Vinogradov (1935) to be associated in one way or another with the fabric of life. From them are derived all the compounds and systems of biology, and, whether the organism be regarded as an episode in the history of matter or of spirit, its behaviour is restricted by the chemical properties of its constituents. Biological elements may be grouped (a) *chemically*, as metals and non-metals; (b) *physiologically*, as essential and non-essential elements; and, (c) *biologically*, as plant and animal constituents.

While these and similar classifications are useful for particular ends, it is simpler at the outset to take the elements in group order according to the periodic table, making a subdivision into *variable* and *invariable* elements, and recognising a quantitative distinction between *primary* elements, *secondary* elements, *micro-constituents*, and *contaminants*.

(1) **Invariable Primary Elements.**—*Hydrogen, Carbon, Nitrogen, Oxygen, Phosphorus.*—These make up the greater part of the organism, representing, individually, from 1 to 60 per cent. of the total weight. They are found in all known forms of life, and determine largely its physical structure.

(2) **Invariable Secondary Elements.**—*Calcium, Magnesium, Sodium, Potassium, Iron, Sulphur, Chlorine.*—These are equally necessary for life, but occur in much smaller quantities, usually from 0.05 to 1 per cent. of the total weight of the organism.

Among the vertebrates, calcium is a primary element.

In many plants, sodium, calcium and iron may be mere micro-constituents.

(3) **Invariable Micro-constituents.**—*Copper, Boron, Silicon, Manganese, Fluorine, Iodine.*—These are believed to be present in all forms of life. The concentrations are minute; usually less than 0.005 per cent. of tissue weight.

Silicon may appear as a secondary element in certain plants and in marine organisms.

(4) **Variable Secondary Elements.**—*Zinc, Titanium, Vanadium, Bromine.*—These elements reach a relatively high concentration in certain species, but their occurrence and importance in other species are often doubtful.

(5) **Variable Micro-constituents.**—*Lithium, Rubidium, Caesium, Silver, Beryllium, Strontium, Cadmium, Germanium, Tin, Lead, Arsenic, Chromium, Cobalt, Nickel, Aluminium, Molybdenum, Barium.*—Some of these have only been detected in a few species, and their functions are obscure.

(6) **Contaminants.**—*Argon, Helium, Mercury, Thallium, Selenium, Bismuth and Gold.*—This classification (Fearon, 1933) includes thirty-nine elements; the additional twenty-one accepted by Vinogradov are mostly what he terms "ultra-micro" elements.

*Biological Elements classified according to their Distribution as
Percentage Body-weight of the Organism*

INVARIABLE (18).			VARIABLE (21).		
Primary (1 — 60%).	Secondary (0.05 — 1%).	Micro- constituents ($< 0.05\%$).	Secondary.	Micro- constituents.	Contaminants (?).
Hydrogen Carbon Nitrogen Oxygen Phosphorus	Sodium Magnesium Sulphur Chlorine Potassium Calcium Iron	Boron Fluorine Silicon Manganese Copper Iodine	Titanium Vanadium Zinc Bromine	Lithium Beryllium Aluminium Chromium Cobalt Nickel Germanium Arsenic Rubidium Strontium Molybdenum Silver Cadmium Tin Caesium Barium Lead	Helium Argon Selenium Gold Mercury Bismuth Thallium
5	7	6	4		7
				17	

Periodic Classification of the Biological Elements.—Chemical elements may be arranged in order of increasing atomic weight (a.w) and atomic number (a.n.), the latter being the value of the nuclear charge as expressed by the number of satellite electrons carried by the atom. The nuclear charge increases with increase in atomic weight, and is indicated by the numerical position of the element in the periodic table. These fundamental properties are denoted by prefixes to the symbol for the element, the upper prefix representing atomic weight and the lower representing atomic number. As, for example, $^{14}_7\text{N}$ denotes nitrogen of a.w. 14 and a.n. 7. In symbols representing chemical compounds, the lower suffix always denotes the number of atoms present. Thus, O_2 represents the oxygen molecule, which is composed of two oxygen atoms.

Biological Elements classified according to Position in the Periodic Table

Group 0	Group I α β H 1	Group II α β 2	Group III α β 3	Group IV α β 4	Group V α β 5	Group VI α β 6	Group VII α β 7	Group VIII α β 8
He 2	Li 3	Be 4	B 5	C 6	N 7	O 8	F 9	
Ne 10	Na 11	Mg 12	Al 13	Si 14	P 15	S 16	Cl 17	
A 18	K 19 Cu 29	Ca 20 Zn 30	Sc 21 Ga 31	Ti 22 Ge 32	V 23 As 33	Cr 24 Se 34 Br 35	Mn 25 I 53	Fe 26 Co 27 Ni 28
Kr 36	Rb 37 Ag 47	Sr 38 Cd 48	Y 39 In 49	Zr 40 Sn 50	Cb 41 Sb 51	Hf 42 Te 52	Mo 43 I 53	Ru 44 Rh 45 Pd 46
Xe 54	Cs 55 Au 79	Ba 56 Hg 80	La 57 Tl 81	Hf 72 Pb 82	Ta 73 Bi 83	W 74 U 92	Re 75	Os 76 Ir 77 Pt 78
Rn 86		Ra 88	Pa 89	Th 90				

The periodic table can be divided into three regions by means of two lines. Invariable biological elements, with the exception of Be, are those above the upper line; variable elements occur between the lines; and ultra-micro and non-biological elements lie below the continuous line.

Isotopes.—Many elements occur in nature as mixtures of two or more closely related forms having the same atomic number and chemical properties, but differing slightly in atomic weight. Thus, chlorine (a.w. 35.5) is a mixture of about 75 per cent. ^{35}Cl and

25 per cent. ^{37}Cl , with a trace of ^{39}Cl . Hydrogen (a.w. 1.0078) is chiefly ^1H , with about 0.02 per cent. ^2H (*deuterium*). These related species are termed *isotopes*, and are defined as elements of similar atomic number but different atomic weight. Including the isotopic forms, over two hundred and fifty different species of atoms are now recognised as represented among the ninety known elements.

Isotopic forms of elements, such as ^{15}N and ^{32}P , are used to trace the course of metabolism in plants and animals (p. 32).

Functions of the Elements.—At least seven different functions may be ascribed to the biological elements:—

(1) *Plastic and Storage Elements.*—Carbon and Nitrogen, which form the framework of the tissue colloids, the *cytoskeleton* of the cell. Sulphur and Phosphorus, which form reactive groups in organic compounds.

(2) *Energy-exchange Elements.*—Hydrogen and Oxygen.

(3) *Skeletal Elements.*—Calcium, Magnesium, Phosphorus, Fluorine, Silicon.

(4) *Electrolytes and Osmotic Regulators.*—Cations of Sodium, Potassium, Calcium, Magnesium; anions of Chlorine, Phosphate and Carbonate.

(5) *Catalytic Elements.*—Components of the oxidation-reduction systems: Iron, Copper, Manganese, Zinc, Iodine, Sulphur. Activators of enzymes: Calcium, Magnesium, Cobalt.

(6) *Replacement Elements.*—Members of which can supplement or substitute other elements.

(7) *Micro-constituents of Unknown Significance.*—Bromine, Nickel, Molybdenum, Silver, Arsenic, Silicon, together with the “ultra-micro” elements.

The chemical constitution of organisms is closely similar with regard to H, C, N, O, S and P, but the occurrence and content of elements such as Fe, Mn, Br, I, B, As, Ti, Va and Zn varies greatly from species to species. Vinogradov has shown that some organisms act as accumulators of specific elements, and thereby modify the surface composition of the earth. Starkenstein (1936) has pointed out that metals of group 5 (As, Sb, Bi) or of period IV (Hg, Pb, Th) are capable of causing chronic poisoning owing to their progressive retention by the organism.

Detection and Estimation of the Elements.—Elements of primary or secondary importance have been found and estimated by the routine procedure of chemical analysis applied to animal and plant ash.

The work has been greatly refined in recent years by the systematic use of the spectroscope (Ramage *et al.*, 1929–1936; Dutoit and Zbinden, 1929, 1930; Webb, 1937).

This enables traces of micro-constituents to be detected and photographed as line spectra.

THE ELEMENTS IN GROUP ORDER

Group I : { **Hydrogen.**
Alkaline metals: Lithium, Sodium, Potassium, Rubidium,
Copper, Silver.

Hydrogen.—H, a.n. 1; a.w. 1.008. A primary element ranking next to oxygen and nitrogen in biological importance. The total H content of an organism depends largely on the water content. The adult human body contains approximately 9.9 per cent. of hydrogen, chiefly as water.

Forms of Occurrence.—Free hydrogen, H_2 , is an end-product of cellulose fermentation, and may appear in the alimentary tract of herbivora, otherwise the free element does not appear to enter into the life-cycle of higher plants or animals. Ionic hydrogen, H^+ , is present in all aqueous solutions, and its concentration determines the degree of acidity. Ionisable hydrogen occurs in all acids and acid salts, of which the carbonates and the phosphates are of special biological importance. Water, $(H_2O)_n$, is the commonest natural form of hydrogen: it makes up 60–80 per cent. of most tissues, and 90–99 per cent. of secretions. Hydrogen peroxide, H_2O_2 , occurs as an intermediate reactant in tissue oxidations. Ammonia, NH_3 , may be included among the inorganic forms of hydrogen, but is more usually studied as a nitrogen derivative. Organic hydrogen is found chiefly in proteins and carbohydrates (7 per cent.), and in fats (12–14 per cent.). To a lesser extent it is present in all bio-organic compounds.

Significance.—Hydrogen is a primary constituent of organic foodstuffs, and constitutes the ultimate fuel of life, as well as possessing the greatest heat of combustion of all the elements. The H-ion, or **Proton**, is the most electro-positive and mobile of all ions. Its concentration qualifies the properties of many tissue constituents, notably colloids and colloidal systems, such as enzymes.

Deuterium.—A heavy isotope of hydrogen, 2H , was discovered in 1932, by H. M. Urey and his colleagues, and subsequently termed *deuterium*, D. Deuterium oxide, D_2O , occurs in all natural waters, the proportion being about 1:6,000 in rain water. This *heavy water* fraction can be concentrated by evaporation, and by fractional electrolysis the H_2O can be removed.

	D_2O	H_2O
Melting point . . .	3.8°	0°
Boiling point . . .	101.42°	100°
Density at 25° . . .	1.105	1.0

When first discovered, it was claimed that pure D_2O is toxic to lower forms of life, and the suggestion was made that the physical changes of old age might be due to accumulation of heavy water in the organism. The alleged toxicity of D_2O has not been confirmed by later workers, and the organism appears to be indifferent to the compound in low concentrations. Thus, Erlenmeyer and Gärtner (1934) found that the animal body does not appreciably change the D_2O content of the water of the diet. By using compounds containing deuterium instead of hydrogen it has been possible to label molecules of fats, sterols and other biological compounds, and trace their fate in the organism (p. 320).

Lithium.—Li, a.n. 3 ; a.w. 6.94. A variable micro-constituent of plants, marine animals, human bone, muscle, and lung tissue. The metal resembles sodium and potassium in its pharmacological effects, but is much more toxic, 0.1–0.2 gm. per kgm. body weight being fatal for dogs and cats. No specific function has yet been assigned to lithium.

In very few of the marine organisms examined by Webb (1937) was the lithium content higher than that of the environment ; the value for sea water being 100–170 mg. Li per cubic metre (Bardet, *et al.*, 1937).

Sodium.—Na, a.n. 11 ; a.w. 23.00. Sodium is probably a universal micro-constituent in plants ; the reported values range from 0.0075–0.15 per cent. of fresh tissue, marine species being richest. In animals the metal is found in much greater amounts, and makes up 0.1–0.5 per cent. of the total body weight of mammals. The concentration is always higher in the extracellular liquids (blood plasma, lymph, digestive secretions, sweat, urine) than in the cells. The value of human blood serum is kept remarkably constant at about 335 mg. Na per 100 ml., whereas the red corpuscles contain only about 0.016 per cent., or 7 mgm. equivalents of Na^+ per litre.

Sea water contains 1.059 per cent. Na.

Sodium values for fresh human tissue, expressed in Na per cent., are : muscle, 0.064 ; kidney, 0.175 ; brain, 0.254 ; lung, 0.312 ; liver, 0.181 ; entire blood, 188 mg. per 100 ml. ; spinal fluid, 290–465 mg. per 100 ml. ; urine, 46–608 mg. per 100 ml. ; cartilage, 2 per cent.

Among organs, the kidney is rich in sodium owing to its power of concentrating the metal during urinary secretion. Tissue cells other than those of the kidney usually have a sodium value below that of their liquid environment ; transudates approximate to the sodium value of blood plasma ; while true secretions, such as milk and urine, may show wide variations in their sodium content. The exceptionally high value for cartilage is explained by Bunge as being

the persistent affinity of this ancient tissue for the characteristic metal of the sea water, which at one time was the cradle of life.

The human excretion of sodium is about 20–30 gm. daily, expressed as NaCl; it comes largely from the condiments used in the diet, and is eliminated to the extent of 95–99 per cent., as a solute in the urine.

Forms of Occurrence.—The concentration of Na-ions in blood is nearly as high as it would be were the metal entirely present as NaCl and NaHCO_3 , from which it is inferred that most of the sodium of the organism is present as a simple ion.

Significance.—Sodium is the chief circulating extra-cellular cation of the animal. Cell membranes, once formed, are relatively impermeable to Na^+ . The solubility of sodium salts favours their uptake, but restricts sodium storage, and the metal must be supplied continually in the animal diet.

Representative values for foodstuffs, expressed as mg. per 100 gm. fresh material are: meat, 65–80; milk, 43; eggs, 185; fish, 125; liver, brain, kidney, 110–160; cereals, 5–30; potatoes, 3–4; green vegetables, 3–15; root vegetables, 10–60; fruits, 0–3; nuts, 2–10.

McCance (1936, 1), from whose paper the above data are taken, observes that a man would have to eat twice his own weight of potatoes daily to obtain his biological requirement of sodium.

Herbivorous animals deprived of sodium develop a characteristic “salt hunger,” attributed by Bunge to the disturbing effect of the excess of potassium present in all vegetables. Benedict (1915) has found 0.2 gm. NaCl *per diem* to represent the minimal requirement of adult man, although this quantity is exceeded ten or twentyfold to allow for loss of the metal by renal excretion.

Forced loss of sodium and chloride evoked in the human subject by an almost salt-free diet, aggravated by sweating, caused cramps, weakness, and severe cardio-respiratory distress on exertion (McCance, 1936, 2).

Special functions ascribed to sodium are:—(1) *A transport solute*. Since most sodium compounds are freely soluble in water, the metal may aid in the solution and distribution of metabolites. (2) *A physiological ion*. Na^+ , along with K^+ , Ca^{++} , Mg^{++} , H^+ and HO^- , is a member of a group of ions necessary for maintaining tissue excitability in higher plants and animals. (3) *A buffer component*. Sodium acid carbonate and sodium acid phosphate form the chief buffer systems whereby the tissues are kept within the normal range of H-ion concentration. (4) *A chlorine carrier*. Sodium chloride is the chief salt of sodium, and this partnership is preserved throughout the kingdom of animal life. It is the principal form in which the metal enters and leaves the organism.

The Na and K content of the blood are regulated by the adrenal cortex. Glandular dysfunction, as seen in Addison's disease, is marked by a characteristic fall in the plasma chloride, and when this is compensated by administration of about 10–20 gm. of additional NaCl *per diem*, the asthenia and vascular disturbances are rectified.

Land plants which have no extracellular circulation may contain almost no sodium, and it has been claimed that insects, such as *Drosophila*, are independent of the metal to such an extent that they can lose 95 per cent. of their total Na without impairment of efficiency.

Potassium.—K, a.n. 19; a.w. 39.10. A primary constituent of all plants; the concentration greatly exceeding that of sodium. Reported values range from approximately 0.1 to more than 1.0 per cent. K in fresh tissue, maximal concentration being found in the regions of active growth, such as leaf tips, secondary roots and pollen tubes.

Potassium is universal in animal tissues, and, unlike sodium, is associated chiefly with cellular structures rather than with fluids. This may be due to the fact that organic derivatives of potassium are less soluble as a class than the corresponding sodium derivatives.

Average values for fresh human tissues, expressed in K⁺ percentages, are: muscle, 0.349; kidney, 0.196; entire blood, 182 mg. per 100 ml.; serum, 19–24 mg. per 100 ml.; corpuscles, 428 mg. per 100 ml.

Sea water contains about 0.38 gm. K per litre.

The total potassium content of higher land animals is usually slightly greater than the total sodium content, being 0.1–0.2 per cent. of the body weight. The internal distribution, however, of the elements is quite different. Potassium, maintaining a vegetable tradition, accumulates in the tissues; sodium, true to a marine ancestry, circulates in the fluids.

Forms of Occurrence.—By cold water extraction, plant tissues can be freed completely from potassium, which indicates that it occurs chiefly in ionic form.

After incineration of vegetable matter, potassium is found abundantly in the ash, almost entirely as the carbonate. After extraction by water and recrystallisation, this provides the "potash" of commerce, and, incidentally, the English name for the metal.

Most of the potassium of higher animals can be extracted in the form of phosphate, but organic compounds are known. The wool fat of sheep is rich in lipide potassium, and is an important channel of excretion of the metal.

Significance.—(1) *A Photosynthetic Factor.*—In the plant, potas-

sium is necessary for chlorophyll manufacture and function, although it appears to be absent from the chloroplast.

(2) *A Factor in Plant Growth*.—Potassium appears to be essential for normal cell division, nitrate reduction, and protein synthesis.

(3) *A Factor in Animal Growth*.—Miller (1923, 1926) reports that growth of young rats ceases when the potassium content of the dietary falls below 0.1 per cent. Adult animals can be kept in health on a dietary containing 2 mg. K *per diem*; young rats require much more, namely, 15 mg. K *per diem*, for males, and 8 mg. *per diem* for females.

(4) *A Physiological Ion*.—In the animal, potassium is the chief metallic ion of the cell interior, and forms one of the group necessary for tissue excitability. The passage of a nerve impulse along a fibre is accompanied by a temporary release of K ions.

(5) *A Diuretic*.—The mammalian kidney is more sensitive to potassium salts than to sodium salts, and potassium administration is followed by a compensatory diuresis.

(6) *A Radio-Active Element*.—Potassium is the most radio active of all biological elements. This is due entirely to the presence of an isotope ^{40}K , which constitutes about 0.01 per cent. of naturally occurring potassium, and which on disintegration yields calcium, beta particles and gamma radiation. The change is of interest because K^+ and Ca^{++} are physiologically antagonistic. Attempts to ascribe the unique biological properties of potassium to its radio-activity are not supported by the work of Glazko and Greenberg (1939), who found neither artificially prepared radio-sodium, ^{24}Na , nor radio-phosphorus could replace natural potassium in maintaining the beat of the isolated heart.

The European mixed dietary provides about 2–4 gm. K *per diem*, which is more than ample for nutritional requirements.

The general biochemistry of potassium has been reviewed by Vanhems (1934).

Copper.—Cu, a.n. 29; a.w. 63.57. An invariable micro-constituent of plants and animals. In plants the copper value is, roughly, one-fifth that of manganese and one-fifteenth that of iron. It ranges from 0.25 mg. Cu per litre of potato juice up to 40 mg. per kgm, in dried lettuce leaf. Leguminous plants and their seeds are rich in the metal.

Representative values, expressed in mg. Cu per kg. fresh edible material, are: almond, 12.1; apple, 0.8; asparagus, 1.4; bean, 6.5; cabbage, 0.5; carrot, 0.8; pea, 2.4; potato, 1.7.

In animals, oysters are exceptionally rich, the value being 24–60 mg. Cu per kg. fresh tissue. Red-blooded fish contain only

1–2 mg. per kg. Higher animals display copper-rich regions (liver, kidney, heart, brain, hair), and copper-poor regions (skin, lung, pancreas, spleen, skeletal muscle). Ramage and the Sheldons find that large stores of Cu accumulate in the foetal liver.

Values for fresh human tissue, in mg. Cu per kg., are : adult liver, 1.3–3.9 ; brain, 3.6–6.0 ; blood serum, 1.7 ; milk, 0.05–0.5 mg. per litre.

Copper is a primary element in the blood of some marine invertebrates, notably the king crab (*Limulus polyphemus*) and the lobster (*Palinurus vulgaris*), where it is part of the blue respiratory pigment, **hæmocyanin**, which is the analogue of the red pigment hæmoglobin, of higher animals. The copper content of sea water was formerly believed to be 100–200 mg. per cubic metre, but Atkins (1932) has shown that 10 mg. per cubic metre is more exact.

Forms of Occurrence.—At least four derivatives are known ; *turacin*, a copper porphyrin found as a purple pigment in feathers ; *hæmocuprin*, obtained from hæmocyanin ; *hæmocuprein*, a copper-protein compound in red blood cells and serum ; and *hepatocuprein*, found in liver (Mann and Keilin, 1938). Less than half the copper in plants occurs in a water-soluble form, which suggests the existence of other organic derivatives.

Significance.—(1) *A Respiratory Pigment.*—Copper as hæmocyanin partakes in oxygen transport in the blood of many arachnids, crustaceans, and molluscs.

(2) *A Feather Pigment.*—No special function has been found for the turacin of feathers. It is water-soluble, and may be a form in which the metal is excreted.

(3) *A Factor in Hæmoglobin Synthesis.*—Copper salts administered in micro-dosage are effective in raising the hæmoglobin content of mammalian blood in some conditions of anæmia. The metal is believed to aid in the synthesis of the porphyrin nucleus of hæmoglobin, and the mobilisation of stored iron.

(4) *A Factor in Plant Growth.*—In optimal minima, copper stimulates growth and germination of many plants, especially *Leguminosæ*. In higher concentrations, it is very toxic to all forms of plant life, especially *Algæ*.

(5) *An Oxidation Catalyst.*—Copper is associated with glutathione in the tissues, and may be necessary for the auto-oxidation of this catalyst.

Dietary sources of copper range from 0.1 mg. Cu per kg. fresh vegetables to 44 mg. per kg. fresh calf liver. The mixed human diet provides 0.1 to 5 mg. Cu *per diem*. Milk is usually very deficient in Cu, and Daniels and Wright (1934) suggest that the diet

of children of pre-school age should contain not less than 0.1 mg Cu per kg. body weight.

Surveys of the copper content of food materials have been made by many workers, notably Lindow *et al* (1929), and Cunningham (1931).

Copper as a Growth-inhibitor of Micro-organisms.—O'Meara and Macsween (1936) have made the observation that the failure of staphylococcus and other pathogenic organisms to grow in culture media may be due to the presence of copper, and recognition of this fact may necessitate a reclassification of bacteria, as many species are identified by their sensitivity to aerobic or other conditions, which in turn may be dependent on the presence of Cu ions. Copper in a culture medium is often due to contamination of the peptone employed in preparing the solution. Serum and other agents, some of which have been supposed to have specific growth-promoting qualities, appear to owe something to their power of protecting micro-organisms from the toxic effect of metals. A high-grade bacteriological peptone contains less than one part of copper per million.

Rubidium.—Rb, a.n. 37; a.w. 85.45. Sheldon and Ramage (1931) find rubidium as a constant micro-constituent in all human tissues, but its occurrence in lower animals and plants is very sporadic. The metal is closely related to potassium, and Rubenstein reports that marine diatoms and possibly some of the seed plants can replace Rb^+ for K^+ in cell growth. Higher animals cannot survive such substitutions. The rubidium content of sea water is assessed by Schmidt at 10–15 mg. per litre, a value that is almost certainly excessive in view of the fact that Ramage finds that marine animals never contain more Rb than 0.002 per cent. of their dry weight.

Silver.—Ag, a.n. 47; a.w. 107.88. Fox and Ramage (1931) find that silver has a wide but very sporadic distribution, maximal values being 0.2 per cent of dry material in fungi, and 0.005 per cent. in the livers of marine lamellibranchs. It is a micro-constituent of the human body, being located chiefly in uterus, ovary and thyroid.

The silver content of sea water is about 20 mg. per cubic metre.

Cesium.—Cs, a.n. 55; a.w. 132.8. A doubtful constituent of the animal body. Sonstadt (1870) reported the presence of Cs in algæ, mollusc shells and sea water, in which Thompson and Robinson (1932) subsequently failed to detect it, using a concentrate from 200 kg.

Group II: $\left\{ \begin{array}{l} \text{Alkaline-earth metals: Magnesium, Calcium,} \\ \text{Strontium, Barium.} \\ \text{Zinc, Cadmium.} \end{array} \right.$

Magnesium.—Mg, a.n. 12; a.w. 24.32. The essential metal of chlorophyll, and, therefore, present in all green plants. It also occurs as a universal micro-constituent of lower plants.

Representative values, expressed in mg. Mg per 100 gm. fresh tissue, are: wheat grain, 129; spinach leaf, 76; carrot leaf, 62; carrot root, 19; potato tuber, 32; lentil, 45; cherry fruit, 20.

Some marine animals are very rich in magnesium, and the skeletons of echinoderms may contain upwards of 10 per cent. MgCO_3 . In these organisms, Mg is being concentrated in preference to Ca, owing to the relatively greater supply available in the environment. The magnesium content of sea water is 0.136 per cent., the metal ranking next to sodium in quantitative importance.

Magnesium is universal in the skeleton, muscles and nerve tissue of higher animals, as shown by the sensitive colorimetric methods now available for its estimation (Cruess-Callaghan, 1936). The distribution of the solute is very uneven, ranging from 2.7 mg. per 100 gm. of plasma to 23 gm. per 100 gm. of skeletal muscle, and up to 50 mg. per 100 gm. of human heart muscle. Magnesium and calcium are closely associated in the skeleton, but not proportionally distributed. Human bone has a magnesium phosphate content of 0.6 per cent. fresh tissue. Human blood serum has an almost constant value of 1.3-5 mg. per 100 ml., and red corpuscles contain 2.3-4 mg. per 100 ml. The urinary content varies widely from about 50 to 250 mg. per litre, the average being 110 mg., which corresponds to a daily intake of approximately 0.2 gm. of the metal, the residue being excreted by the intestine. The chief source in the diet is the chlorophyll of the vegetable food-stuffs. Human milk contains about 6 mg. per 100 ml.

Forms of Occurrence.—(1) Accompanying, supplementing, or replacing calcium as basic phosphate and carbonate in skeletal tissue.

(2) The acid phosphate, MgHPO_4 , in urine and in urinary precipitates of "earthy phosphates."

(3) As a porphyrin derivative in chlorophyll.

(4) As inositol hexaphosphate in *phytol*, which forms the coating of seeds.

(5) As an unidentified micro-constituent of tissues and secretions.

Significance.—(1) *A Structural Element.*—Magnesium, like calcium, aids in forming the inorganic matrix of the animal skeleton.

(2) *A Transport Element.*—Magnesium aids in phosphate transport and excretion in animals.

(3) *A Physiological Ion.*— Mg^{++} and Ca^{++} are the chief divalent ions that regulate tissue excitability. The effect is complex. In muscle, they are complementary to a limited extent, and can antagonise K^+ . In nerve, Mg^{++} and Ca^{++} are antagonistic; Mg^{++} has a specific depressant action, partly neutralised by Ca^{++} . Intravenous injection of sufficient Mg to raise the level in the blood up to 0.02 per cent. results in deep anaesthesia and paralysis

of the voluntary muscles, an effect that is immediately abolished by the injection of a corresponding amount of a soluble Ca salt.

(4) *An Enzyme Activator*.—Mg ions are necessary for the phosphatase enzymes of the acid type found in kidney, bone, intestine, and in the mammary gland and the blood plasma (p. 216); and the metal is necessary also for the activation of the glycolytic enzyme system of muscle (p. 293).

(5) *A Factor in Plant Growth*.—In the absence of magnesium, green plants are unable to manufacture chlorophyll, and fail to grow. It is the catalytic metal in photosynthesis.

(6) *A Factor in Animal Growth*.—Rats and dogs on a diet deficient in magnesium develop epileptiform convulsions and die. The condition is specific, and independent of the calcium level in the blood, and, according to Brookfield (1934), is due to renal and hepatic dysfunction. These may arise from failure of the phosphatase enzyme systems owing to lack of the specific activator.

Calcium.—Ca, a.n. 20; a.w. 40·07. Calcium is invariably present in plants, and is essential for growth of all vegetation, with the possible exception of some lower forms. The content ranges from about 10–100 mg. Ca per 100 gm. fresh tissue. It is high in cereal grain.

Average values, in mg. Ca per 100 gm. fresh edible tissue, are: asparagus, 28; beetroot, 21; cabbage leaf, 119; lettuce leaf, 35; onion bulb, 42; potato tuber, 11; oat grain, 117; barley grain, 86; wheat grain, 90.

The values show marked differences depending on species and soil conditions. In animals, calcium is universal, and occurs in skeletal and soft tissues.

The human body contains 2–2·5 per cent. Ca, 99 per cent. of which is located in the skeleton. Vertebrate bone contains about 10 per cent. Ca, recoverable as tricalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, calcium carbonate, CaCO_3 , and calcium fluoride, CaF_2 . Fresh vertebrate tissue has a calcium value ranging from about 6 mg. Ca per 100 gm. in muscle, up to 20 mg. in kidney, and 34 mg. in thyroid. Milk is very rich; average values being 30–80 mg. for human milk, and up to 120 mg. Ca per 100 ml. for cow's milk. Consequently, milk and cheese are most important dietary sources of calcium. The output in human urine is very variable, ranging from 0·5 mg. to more than 50 mg. per 100 ml. The average for twenty-four hours is 200 mg., corresponding to a usual calcium intake of 1·0 gm. of the metal, 65–80 per cent. of which is excreted by the intestine.

Sherman emphasises the necessity for an adequate intake of calcium in the human diet, especially in the nutrition of children, and places the daily requirement at 0·75–1·0 gm.

Forms of Occurrence.—(1) *Insoluble Salts.*—Tricalcium phosphate, calcium carbonate, calcium fluoride. In man, 84–90 per cent. of bone calcium is present as phosphate; the rest is chiefly carbonate. Calcium fluoride is the characteristic salt of tooth enamel. Insoluble calcium soaps form most of the faecal calcium, especially when the diet is rich in fats.

(2) *Soluble Salts.*—Calcium acid phosphate, CaHPO_4 , and calcium acid carbonate, $\text{Ca}(\text{HCO}_3)_2$, carry the metal in the blood, tissues, and urine. Soluble protein derivatives also are known, such as the calcium caseinate of milk.

(3) *Blood Calcium.*—Calcium is almost absent from the red blood cells, but exists in the serum in colloidal and non-colloidal forms. For human serum the average total value is 9–11 mg. Ca per 100 ml., about 6 mg. of which are non-colloidal and diffusible. Charles (1931) reports a sex difference in serum calcium, the female value in lower animals being 3–22.5 per cent. greater than the male value. In birds this may be due to mobilisation of the salts for the egg shell.

Significance.—(1) *A Regulator of Soil Acidity.*—Calcium carbonate is one of the natural factors concerned in reducing the acidity of soil, and is used for this purpose in agriculture.

(2) *A Plant Structural Constituent.*—Calcium occurs as a pectate in the cell walls of plants, especially fruits, and imparts rigidity.

(3) *An Animal Structural Constituent.*—Calcium is the characteristic metal of the animal skeleton, although it may be represented in part by magnesium and entirely by silicon in some lower forms of life. Skeletal tissue, in addition to its obvious mechanical functions, provides a vast but somewhat inaccessible reserve of calcium.

(4) *A Physiological Ion.*—In plants, Ca^{++} tends to antagonise the toxic effects of K^+ , Na^+ , and Mg^{++} . In animals, an increase in the concentration of Ca^{++} (or Mg^{++} , or H^+) within critical limits tends to depress neuro-motor excitability and nerve conductivity.

(5) *A Factor in Muscle Contraction.*— Ca^{++} in optimal concentration is necessary for muscle contraction. Excess causes increase in tone, leading to “calcium rigor.” The effect is antagonised by K^+ , and appears to be independent of the general depressant action of Ca^{++} on the neuro-muscular junction.

(6) *A Factor in Blood Coagulation.*— Ca^{++} participates in the normal coagulation of shed blood. It can be replaced, partially, by the related metals, Mg, Sr and Ba. Given by mouth, calcium salts have no effect on coagulation time in normal subjects.

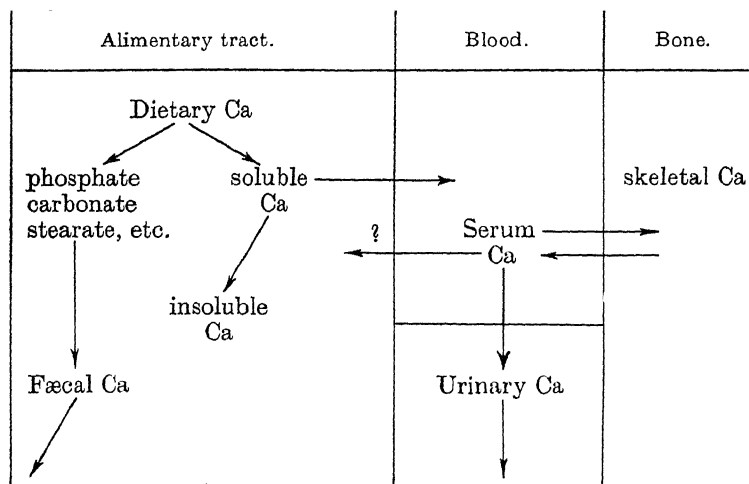
(7) *A Factor in Milk Coagulation.*—Caseinogen contains about 1 per cent. Ca. Under the influence of rennin, caseinogen is converted into a form that reacts with the other calcium salts of the

milk serum, and gives rise to the insoluble casein. Hence, milk curd and cheese are relatively richer in calcium than the original milk.

Determinants of Calcium Metabolism.—In higher animals three factors operate: (1) *the parathyroid hormone, parathyrin*; (2) *the D vitamins*; (3) *sunlight or ultra-violet irradiation*. Parathyrin given orally or intravenously raises the serum calcium until it reaches the level of pathological hypercalcaemia, characterised by vomiting, coma, and cardiac failure. Parathyroidectomy causes a fall in serum calcium until the lower level of 7 mg. Ca per 100 ml. is reached, when the syndrome of hypocalcaemia appears, characterised by tetany.

Vitamin D has little influence on the serum calcium level in health, but in conditions of hypocalcaemia it raises the calcium and phosphate content of the serum, and promotes intestinal absorption of phosphorus and calcium. Solar and ultra-violet irradiation act by enabling the organism to manufacture vitamin D from its sterol precursor, if already present in the tissues.

"There can be no doubt that the development of satisfactory methods of eliminating Ca and Fe with insoluble salts must have been essential for evolutionary survival. Thus, loss of shell is a frequent event in the evolution of some invertebrate phyla, and must have thrown a heavy strain on the excretory mechanism. . . . Man would not have evolved had not the early mammals acquired the property of excreting unwanted calcium in an insoluble form by the gut, where it could not cause mechanical obstruction" (McCance, 1936, 1).



Calcium Metabolism in Man.—Dietary calcium may be (i.) absorbed into the blood stream as soluble calcium, (ii.) precipitated in the alimentary tract as calcium soap, (iii.) left unchanged as phosphate and carbonate.

Serum calcium may be (i.) precipitated as skeletal calcium, (ii.) excreted as urinary calcium, (iii.) reabsorbed into the intestine, (iv.) excreted in the bile.

FACTORS REGULATING CALCIUM METABOLISM

Renal Ca Output increased by :

- (1) Excess Ca in diet.
- (2) Deficit of P in diet.
- (3) Acid dietaries.
- (4) Starvation.
- (5) Parathyrin injection.
- (6) Any other condition tending to raise the serum Ca level.

Intestinal Ca Output increased by :

- (1) Excess Ca in diet.
- (2) Excess of P in diet.
- (3) Alkaline dietaries.
- (4) Excess of fat in diet.
- (5) Rickets and osteomalacia.

According to McCance, calcium and magnesium are not excreted into the intestine, in the human subject.

Skeletal calcium exists in the form of the complex salt, $\text{CaCO}_3 \cdot 3\text{Ca}_3(\text{PO}_4)_2$, and is not in simple equilibrium with the serum calcium. Calcification is a process involving the coaction of several factors, one being the phosphatase enzyme present in plasma and cartilage, another being the maintenance of the optimal calcium-phosphate ratio in the plasma, which normally is such that $[\text{Ca}] \times [\text{PO}_4] = 36$, both being expressed in mg. per 100 ml. of blood.

Strontium.—Sr, a.n. 38 ; a.w. 87.63. Fox and Ramage were the first to show that strontium is generally distributed in marine animals, and their work has been confirmed by Webb. Strontium is the fifth most abundant metal in sea water, the concentration being about 13.5 mg. per litre.

Strontium has also been found in the ash of marine plants, and as a micro-constituent in the animal skeleton, the liver and the lung of the human foetus. Its chief location appears to be the retina. No significance has yet been ascribed to it.

Barium.—Ba, a.n. 56 ; a.w. 137.4. Owing to the low solubility of barium sulphate, the maximum concentration of Ba ions in sea water is 0.48 mg. per cubic metre. The metal was detected in foraminifera by Schultze, and has been found by Webb in about half the marine organisms examined (1937). Ramage and Sheldon report its presence in the choroids of cattle, but not in those of a wide selection of other vertebrates.

Barium salts are toxic to the higher animal, and the metal was unexpectedly found in the Brazil nut by Seaber (1933) during an investigation of an obscure outbreak of food poisoning.

The metal is concentrated in the husk, or inner part of the testa,

where it makes up 8 per cent. of the total ash. Barium has several times been found in the ash of terrestrial plants, and may substitute other alkaline earth metals.

Zinc.—Zn, a.n. 30 ; a.w. 65·37. A universal micro-constituent. Among plants, values range from less than 1 mg. Zn per kg. fresh tissue in fruit pulp, up to 10 mg. per kgm. in leaves rich in chlorophyll, such as lettuce, cress, spinach, dandelion. Marine animals show values ranging from 3·5 mg. per kg. in elasmobranchs up to 188·5–341 mg. in oysters. About half of the total zinc in oysters can be removed as a simple solute, by dialysis. The 70 kg. human body contains on an average 2·2 gm. of zinc, most of which is in bone and hair. The ordinary mixed diet supplies about 12 mg. Zn daily.

Values, in terms of mg. per kg. fresh tissue, are : liver, 10–76 ; brain, 8–10 ; muscle, 5–10. Thyroid and ovary are specially rich. The metal is constantly present in human and cow's milk, in egg yolk, and in some snake venoms.

The zinc content of sea water is 2–60 mg. per cubic meter.

Significance.—Zinc in low concentration stimulates plant growth, especially that of fungi and cereals. In higher concentration, over 1 : 100,000 ZnSO_4 , it is very toxic to most plants, and can inhibit completely sugar fermentation by yeast.

(1) Stirn, Elvehjem, and Hart (1935), and Bertrand and Bhattacharjee (1935) have shown that rodents fed on a diet deficient in zinc, though adequate in other respects, do not grow to maturity.

(2) It has been shown that, if zinc is present, the hypoglycæmic action of protamine-insulin or spermine-insulin compounds (which are more effective than pure insulin) is greatly prolonged. Insulin preparations contain zinc as a micro-constituent.

(3) The distribution of zinc and of vitamin B_1 run parallel in many natural foods, and the metal may be associated in some way with the action of the vitamin (p. 252).

(4) Bertrand and Vladesco have shown that the accessory glands of the male genital apparatus in mammals are rich in zinc, and the seminal fluid even richer, and suggest that the metal may play a part in fertilisation.

(5) Delezenne (1919) held that the power of snake venom to bring about rapid hydrolysis of nucleoproteins and phosphatides was correlated with its large zinc content. Cristol (1923) was led to conclude that this property of zinc enabled it to act as a necessary catalytic agent for the chemical changes that take place at mitosis. He observed that in malignant tumours or in the blood and liver in cases of leucocythæmia—both tissues in which mitosis is pro-

ceeding very rapidly—the zinc content was from 3 to 18 times the normal. Cruickshank (1936) has surveyed the sources and distribution of zinc in relation to human nutrition, and claims that tuberculosis is associated with a zinc deficiency, and malignant conditions with chronic zinc poisoning. Beri-beri may be due partly to zinc deficiency (Eggleton, 1939). The enzyme carbonic anhydrase is a zinc protein complex.

Cadmium.—Cd, a.n. 48; a.w. 112.4. This metal is usually associated in nature with zinc, and has been detected spectroscopically only in the liver of marine molluscs. Its significance is unknown.

Group III: Boron, Aluminium.

Boron.—B, a.n. 5; a.w. 11. This non-metal has been detected in all plants examined. The value is least in cereals (the ash of which contains about 0.5 gm. boric acid, H_2BO_3 , per kg.), high in the beetroot, and maximal in the date fruit (30 mg. B per kg. pulp). Grape vines and wine are rich in boron.

Sea water contains about 4.5 mg. B per litre. Borates must form an important part of the buffer mechanism of sea water, being second only to carbonates, and exceeding the combined effects of phosphates, arsenates and silicates. Webb finds B constant in marine organisms, the value being approximately that of the environment. There is little tendency to accumulate or exclude the element. Its concentration in higher animals is low and uncertain; the mammalian value being about 0.01 mg. B per kg. fresh tissue, or less. It is not a normal micro-constituent of milk, but may be introduced as a preservative.

Significance.—Boron is necessary for the development of higher plants, and appears to be associated with carbohydrate translocation (Dennis, 1937). In concentrations above 1:100,000 it retards growth. Fungi and certain green algae are extremely inert towards boron, and the toxic action of the element may be used to differentiate them from the relatively susceptible bacteria. The absence of boron from milk suggests that the element is not essential for the higher animal. Given in small doses for long periods it has no obvious effect on growth or metabolism. Large doses are toxic, and cause gastro-intestinal disturbance, inhibition of dermal secretion, and loss of hair.

Aluminium.—Al, a.n. 13; a.w. 27.1. This metal, abundant as aluminium silicate on the surface of the earth, is relatively rare in the organism.

It is present in all plants, being maximal in some mosses, which, according to Vinogradov, act as Al accumulators.

The value for fresh vegetable foods ranges from 35 mg. Al per kg., in cherries, and 40 mg. in onions, down to less than 1 mg. in apples and oranges. Green leafy vegetables have a value of 10–20 mg. per kg.

Aluminium is an occasional if not a frequent micro-constituent of animals.

Values reported are: dog's blood, 0.23 mg. per 100 ml.; human

liver, 0.1–1.2 mg. per 100 gm.; human kidney, 0.13–0.87 mg. per 100 gm. Lung tissue, especially from older animals, is relatively rich in the metal.

After oxygen and silicon, aluminium is the most abundant element in the earth's crust, and all clay, dust and detritus is liable to introduce both Al and Si as contaminants into the organism. McCollum has shown that the skin, lungs and intestinal mucosa contain at least twice as much Al as the other organs, which as a rule have an Al value of less than 0.5 parts per million of fresh tissue.

The value for sea water is provisionally assessed at 0.6–2.4 mg. Al per litre.

Significance.—Considering its natural abundance, the very low concentration of aluminium in living organisms is remarkable, and, in consequence, the metal is not regarded as necessary for growth or function. There is no conclusive evidence that small doses of aluminium over long periods have any effect on the growth or health of higher animals, but the subject is still disputed. The lethal dose of the sulphate or the chloride for rats, rabbits, and guinea-pigs is 5–8 gm. per kg. body weight, injected subcutaneously. Resulting changes are degeneration of the renal tubules, necrosis and liver atrophy. Spira (1933) believes that chronic poisoning is liable to occur owing to the careless use of aluminium cooking utensils, but this is deprecated by Monier-Williams (1935).

Group IV : { Carbon, Silicon, Titanium.
 { Germanium, Tin, Lead.

Carbon.—C, a.n. 6; a.w. 12.

The twelfth element in order of abundance on the surface of the earth. It occurs chiefly in oxidised forms, as CO_2 in the atmosphere, H_2CO_3 in the ocean and other waters, and as insoluble carbonates in rocks and soil.

Carbon is the characteristic element of organic material, and accounts for 15–20 per cent. of the total weight of higher organisms. Englia has estimated that about one-thousandth part of the carbon of the earth's surface is in biological use. It occurs, united to hydrogen, in all bio-organic compounds, notably, the three great families of lipides, carbohydrates, and proteins.

Characteristics of Carbon.—The dominant position of carbon in the fabric of life is due to unique chemical properties having biochemical consequences, and it has even been suggested by Jeans that life organisation is a latent property of carbon, which is *biogenic* in the sense that iron is magnetic or radium is radio-active.

(1) *Electro-chemical Character.*—Carbon comes midway in the periodic table, and has the property of uniting with all other known elements (except the members of the argon group). It is able to form stable compounds with strongly electro-positive elements, such as hydrogen, and with strongly electro-negative elements, such as chlorine.

(2) *Tetravalency*.—A carbon atom can unite with four monovalent elements or their equivalent.

(3) *Mutual Combination*.—Carbon atoms freely unite with each other in various ways :—

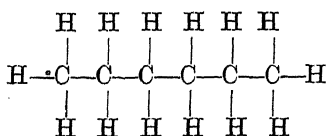
By single-bond linkage, as in *ethane*, $\text{H}_3\text{C}—\text{CH}_3$;

By double-bond linkage, as in *ethylene*, $\text{H}_2\text{C}=\text{CH}_2$;

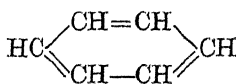
By triple-bond linkage, as in *acetylene*, $\text{HC}\equiv\text{CH}$.

Double and triple-bond linkages are said to be *unsaturated*, and are less stable than single-bond linkages.

(4) *Chain Formation*.—Carbon atoms may unite to form straight, open, or linear chains ; and closed, cyclic chains, or rings. For example :—



Hexane, C_6H_{14} .
(A linear hydrocarbon.)



Benzene, C_6H_6 .
(A cyclic hydrocarbon.)

Cyclic compounds as a type are more stable than linear compounds, and many biochemical reactants are able to alternate between linear and cyclic forms.

(5) *Group Formation*.—Individual grouping tends to persist in organic molecules ; and *radicles* or particular groups of atoms may retain their identity through a series of reactions affecting other parts of the molecule.

These and other properties make possible the existence of a vast community of carbon compounds, which displays :—

(a) *Variety*.—At least a quarter of a million organic compounds are known.

(b) *Complexity of structure* unrivalled among inorganic compounds.

(c) *Family characteristics* due to presence of specific radicles.

(d) *Type reactions*, by means of which particular groups can be identified.

(e) *Isomerism*.—Organic compounds may be so complex that a simple molecular formula is insufficient to distinguish them. For example, $\text{C}_3\text{H}_6\text{O}$ may represent acetone ($\text{CH}_3.\text{CO}.\text{CH}_3$), acetaldehyde ($\text{CH}_3.\text{CH}_2.\text{CHO}$), or allyl alcohol ($\text{CH}_2 : \text{CH}.\text{CH}_2.\text{OH}$). These compounds are *isomers* in that they contain similar sets of atoms arranged differently. Isomerism is a feature of the great family of carbohydrates.

(f) *Low Reactivity*.—Organic compounds tend to react more

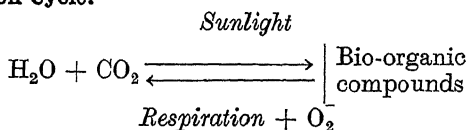
slowly than inorganic compounds, and thus fix a chemical limit to the rate of living.

(g) *Insolubility*.—With the notable exceptions of lower alcohols, acids, amines, and heavily hydroxylated compounds, such as sugars; the majority of organic substances are sparingly soluble in water, although usually soluble in organic solvents such as ether, alcohol, chloroform, and benzene.

Recognition of Carbon Compounds.—(1) If charring occur on heating the solid substance, carbon is present.

(2) Every carbon-containing substance (except CO_2) may be burned in oxygen, and CO_2 is always one of the products of combustion.

The Carbon Cycle.



Silicon.—Si, a.n. 14; a.w. 28.3. The most abundant element in nature, next to oxygen. Aluminium silicate is the chief constituent of clay, and silicates as a class make up 55 per cent. of the earth's crust. Silicon is universal in plants, especially the stems of cereals, bamboo, and coarser grasses. Expressed as percentage SiO_2 in total ash, representative values are: wheat, 2.28; barley, 22.3; oats, 42.64. Potato tuber ash contains about 2 per cent., the bulk being in the skin. Silica, SiO_2 , is the characteristic structural component in the skeletons of silicious sponges. The Si content of sea water is very variable, and ranges from 40 mg. up to 2,000 mg. per cubic metre, the higher values being obtained in shallow water containing suspended silicate.

In higher animals, it is a universal micro-constituent, especially in connective tissue. Average values range from 0.1–0.35 mg. SiO_2 per gm. fresh material. Silicotic human lung may have a value as high as 123 mg. per 100 gm. dry tissue.

Forms of Occurrence.—Skeletal silicon is found as an opaline silicate. Vegetable silicon appears to be a derivative of cellulose or other polysaccharides. In higher animals, the only compound identified is a sterol silicate found in bird's feathers.

Significance.—(1) *A Structural Element.*—Silica confers rigidity to the stems of cereals and grasses, but other factors also assist. It is the supporting element in the skeletal tissue of unicellular marine organisms, and may serve a special purpose in photosynthesis on account of its great transparency. The association of silica with

connective and scar tissue has led to its application in the treatment of diseases. The association is also seen in *silicosis*, a characteristic fibrosis of the lung due to inhalation of silica dust (King and Belt, 1938).

(2) *An Anti-Parasite*.—There is some evidence that silica aids in the protection of cereals against fungi, rust, and other infections.

(3) *A Replacement Element*.—Silicon is a non-metal closely related to carbon, and silico-organic compounds might be expected to appear in place of some carbon compounds, especially in plants.

Titanium.—Ti, a.n. 22 ; a.w. 46.1. An obscure micro-constituent of plants and higher animals. Fish, including mackerel, carp, herring, whiting, have values from 0.3 mg. Ti to 0.9 mg. per kg. fresh tissue. Mammalian liver has an average value of 0.6 mg. per kg. The metal has been detected spectroscopically in human lung tissue and blood ash. Webb has shown that serious errors may arise from the use of carbon electrodes contaminated by traces of titanium which are unmasked by the alkalis in tissue ash. For this reason, many claims as to the distribution of titanium require confirmation.

Germanium.—Ge, a.n. 32 ; a.w. 72.5. Traces of this rare metal have been detected in the ash of some seaweeds, and in blood. Steinberg (1939) claims that traces of Ge are necessary for growth of common moulds.

Tin.—Sn, a.n. 50 ; a.w. 118.7. Tin has been detected in most tissues of the higher animal, especially human brain, spleen, and thyroid. The amount present is very small and may be due to food contamination.

The most reliable estimations of tin in organisms are the analyses of Orton (1924), who found from 10 to 55 parts per million of fresh weight in oysters (the higher figure only for green specimens), and of Bertrand and Ciurea (1931), who found 0.4 to 4 parts in most mammalian organs, but up to 26 parts in the lingual mucosa.

Lead.—Pb, a.n. 82 ; a.w. 207. Traces of lead occur in plants, especially grasses, grown on plumbiferous soils. It is present in many marine animals, especially corals, crustacea, and molluses, and is an erratic micro-constituent of higher animals, appearing inconsistently in human tissues in a manner that suggests lack of specific physiological function (Minot, 1938).

Group V : { **Vanadium.**
Nitrogen, Phosphorus, Arsenic.

Vanadium.—V, a.n. 23 ; a.w. 51. This rare metal is a characteristic constituent of the blood of some marine animals (holothurians and ascidians), where its significance is unknown. It has been found in timber-ash, and as a micro-constituent of animal tissues, and tends to accumulate in the liver, where it may promote oxidation of phospholipides (Bernheim).

Nitrogen.—N, a.n. 7 ; a.w. 14.01. Nitrogen has a very low affinity for other elements, and consequently most of the nitrogen of the environment occurs in a free state in the atmosphere.

BIOLOGICAL

Inorganic nitrogen as nitrate is widely distributed on the surface of the earth, and forms vast deposits in arid regions, as in Chile.

Nitrogen occurs in all living organisms, mostly as a constituent of protein, and is found in substances of organic origin in soil and sea water.

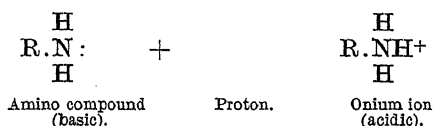
Forms of Occurrence.—The chief biochemical compounds of nitrogen are :—

A. *Inorganic* : NH_3 , $\text{NH}_2\cdot\text{OH}$ (hydroxylamine), HNO_2 , HNO_3 , and their salts.

B. *Simple organic* : HCN , $\text{HO}\cdot\text{CN}$ (cyanic acid), $\text{HS}\cdot\text{CN}$ (thiocyanic acid), CON_2H_4 (urea), $\text{R}\cdot\text{NH}_2$ (amines), $\text{R}\cdot\text{CO}\cdot\text{NH}_2$ (amides), $\text{R}(\text{NH}_2)\cdot\text{COOH}$ (amino acids), $\text{R}\cdot\text{NH}\cdot\text{CO}\cdot\text{NH}_2$ (ureides), $\text{R}\cdot\text{NH}\cdot\text{C}(\text{NH})\cdot\text{NH}_2$ (guanidines).

C. *Complex organic* : purines, pyrimidines, porphyrins, proteins, etc.

Many important biological properties reside in the amino group, NH_2 . Nitrogen is trivalent, and when these valencies are satisfied still possesses a "lone pair" of orbital electrons which are able to accept a proton, or H -ion, thus conferring a positive charge on the group and making it a potential H -ion donator.

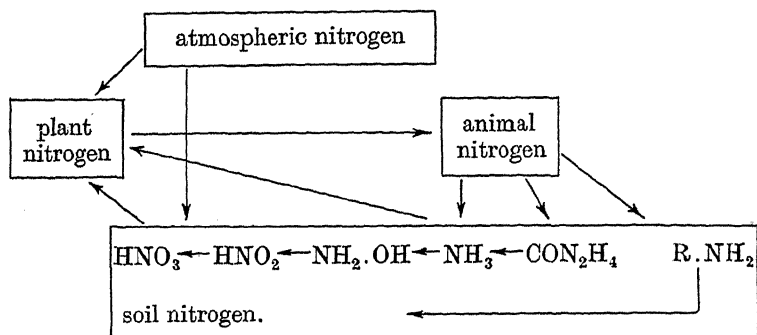


The Nitrogen Cycle.—Nitrogen leaves the atmosphere both directly and as oxides of nitrogen. The oxides are formed in the upper reaches of the atmosphere by photo- and electro-oxidation, and, dissolved in rain, reach the sea and soil as nitrous and nitric acids.

Free atmospheric nitrogen is assimilated by soil organisms, by the root-nodules of leguminous plants (peas, beans, and clover), and possibly by the roots and leaves of cereals. Thus are made available for life several highly reactive forms of nitrogen in various degrees of oxidation and reduction, ranging from the strong acid, NHO_3 , to the base, NH_3 . From these, the plant synthesises proteins and other nitrogen compounds which may be transmitted to the animal, or back again to the soil. The higher animal excretes its waste nitrogen as urea, which is rapidly attacked by micro-organisms, and converted into ammonia, thus enabling the nitrogen to continue in circulation. Among birds and reptiles, waste nitrogen is excreted as purines, principally uric acid, which is a much more stable and less soluble compound than urea. By this process, nitrogen has

been temporarily diverted to form the great guano deposits of the Pacific Islands.

THE NITROGEN CYCLE



Phosphorus.—P, a.n. 15 ; a.w. 31·04. An essential constituent of all plants, the normal range being from 0·1–0·8 per cent. of dry tissue. The value is usually less than that for calcium, but more than that for magnesium or sulphur. The phosphorus content tends to increase with progress up the evolutionary scale. The adult human body contains about 1 per cent., or, approximately, 700 gm. of phosphorus. Of this, 600 gm. are in the skeleton, 57 gm. in muscle, 5 gm. in nerve tissue, and 2 gm. in the blood. The phosphorus content of milk varies greatly with species of animal, and rate of formation of the young skeleton ; average values are : human milk, 0·05 ; cow's milk, 0·18 ; expressed as percentage phosphoric acid. The daily output of the human adult varies from 0·3 gm. P to 2·0 gm., two-thirds of this is excreted by the kidney. The value for inorganic urinary phosphorus ranges, in man, from about 30 to 200 mg. per 100 ml., the average being 80 to 90 mg. Organic urinary phosphorus ranges from 0·7 to 18 mg., the average being 6 mg., representing about 20 mg. H_3PO_4 .

Forms of Occurrence.—(1) *Inorganic.*—Phosphates may occur as electrolytes in soft tissues and salts in skeletal structures. Salts of pyrophosphoric acid ($\text{H}_4\text{P}_2\text{O}_6$) are present in muscle and in yeast. Many of these phosphates are in union with organic compounds, and cannot be classified, strictly, as "inorganic phosphate."

(2) *Organic.*—Glycerophosphates and hexosephosphates ; phosphatides ; phosphoproteins, and unidentified compounds. In plants, phosphorus occurs in phosphoproteins and in *phytin*—the Ca Mg salt of inositol-phosphoric acid. Various forms of muscle and blood phosphates are recognised analytically in animal tissues.

Significance.—Phosphorus has a diversity of independent physiological functions, and the element can be mobilised and distributed in accordance with local needs.

Phosphates act as the transport agents in metabolism, and by forming labile esters, controlled by the phosphatase enzymes, they convey glycerol and simple sugars from the intestine to the blood, transfer calcium to and from the skeleton, carry lipides to the tissues, and participate in the carbohydrate metabolism of muscle, liver and other organs.

(1) *Structural Phosphate*, including phytin, in plants, and bone, in animals.

(2) *Labile Phosphate of Carbohydrate Metabolism.*—Hexose phosphates formed during sugar degradation or polymerisation.

(3) *Labile Phosphate of Lipide Metabolism.*—This participates in transport and degradation of aliphatic acids.

(4) *Labile Phosphate of Muscular Contraction.*—Phosphagens, including phosphocreatine, phosphoarginine and adenosine triphosphate.

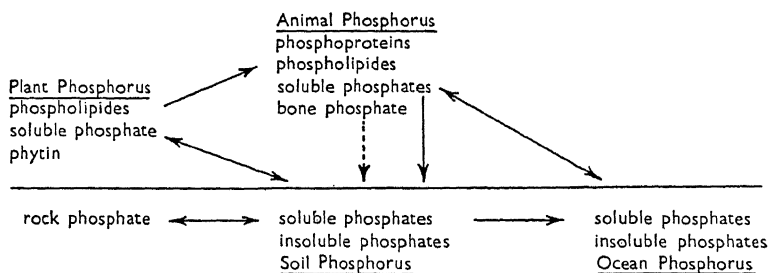
(5) *Lipide Phosphorus* of cell architecture.

(6) *Nuclear Phosphorus.*—The nucleoproteins that form the framework of chromatin and the carriers of heredity.

(7) *Buffer Phosphates*, stabilising the H-ion concentration of cells and tissue fluids.

(8) *Phosphoproteins*, such as casein, provide the chief source of phosphorus for young mammals, and an important *acidogenic* factor in the diet.

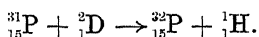
The Phosphate Cycle.—Phosphorus circulates in the kingdoms of life in the oxidised form of phosphate. Plants elaborate soil phosphate into phospholipides and other phospho-compounds, which are transformed by the animal into phosphoproteins and tissue phosphates, the residue being returned to the soil or the sea as metallic phosphate. Soil phosphate is one of the important limiting factors in plant growth, and its provision is one of the tasks of applied agriculture.



The phosphate content of sea water is very variable, and ranges from almost zero, on the surface, to 0.004 per cent., expressed as PO_4 .

By supplying a phosphate containing the radio-active isotope, ^{32}P , and tracing its fate, Hevesy has shown that phosphates are in a state of circulation among the tissue compounds of plants and animals. In five days, 22 per cent. of the phosphate in the animal diet has passed through the organism and been excreted by the kidneys, and 16 per cent. has been excreted by the urine (1935).

The isotope may be obtained by bombarding red phosphorus with deuterium ions, using a cyclotron.



Arsenic.—As, a.n. 33 ; a.w. 75. Salts of arsenic are highly poisonous, and the metal is chiefly of toxicological interest. Small traces occur in a few plants, especially grasses grown on arsenical soils.

Arsenic occurs in sea water to the extent of about 11 to 20 mg. As per cubic metre, and has frequently been included in phosphate estimations by mistake (Atkins, 1927).

In mammals, the distribution is so minute and variable that it can have little biological significance. Impure table salt is a source of arsenic in human tissues. The metal tends to accumulate in skin and hair.

Group VI **Chromium, Molybdenum.** **Oxygen, Sulphur.**

Chromium.—Cr, a.n. 24 ; a.w. 52.0. Traces of this metal have been detected spectroscopically in mammalian blood and tissue ash, especially thyroid and spleen, but, as Webb points out, these data, when obtained by the use of graphite electrodes, are open to question. Chromium salts are very injurious to the kidney, and the biological occurrence of the metal is probably adventitious.

Molybdenum.—Mo, a.n. 42 ; a.w. 96. According to ter Meulen (1931) Mo occurs in nearly all tissues, the maximum being 9 parts per million of dry weight in plants, and 1.5 in animals. His spectrographic technique has been adversely criticised by Dingwall and others (1934), who find that the Mo content of plant tissues depends entirely on the environmental supply.

Oxygen.—O, a.n. 8 ; a.w. 16.00. The most abundant and widely distributed of all elements. It constitutes about one-half of the earth's crust, and about two-thirds of plant and animal tissues. It is an essential element in almost every bio-organic compound, and is necessary, directly or indirectly, for the respiration that accompanies all life. Higher organisms are *aerobic*, and need a continual supply of free, molecular oxygen, although some, when compelled, can go into a temporary state of "oxygen-debt."

Some lower organisms, chiefly bacteria, are *anaerobic*, and can only utilise oxygen in combined forms. A few, the *obligatory anaerobes*, are poisoned by free oxygen.

Significance.—Oxygen is unique in being the only element assimilated in free, molecular form by the animal. Within the organism, it serves to liberate energy. The majority of chemical processes in life are oxidations, and the work performed is derived ultimately from energy obtained by oxidation.

Sulphur.—S, a.n. 16 ; a.w. 32.06. In plants, sulphur is universal and fairly uniformly distributed as proteins, organic sulphides, and inorganic sulphates. Values range from about 0.01 to 0.2 per cent. of fresh material. In higher animals, the sulphur value depends largely on the amount of scleroprotein present. Thus, mammalian muscle has about 0.2 per cent. total sulphur ; skin, hair, feathers, and other epidermal structures may have up to ten times as much. The sulphur content of foods has been surveyed by Masters and McCance (1939).

The total sulphur of human urine ranges, normally, from 30 to 300 mg. per 100 ml., according to the diet, the average being about 90 mg., expressed as H_2SO_4 .

Forms of Occurrence.—(1) *Inorganic Sulphates.*—Salts of H_2SO_4 and alkaline metals, found in almost all plant and animal tissues. They are a form in which sulphur is distributed and excreted.

(2) *Inorganic Sulphides.*— H_2S and alkaline sulphides are products of bacterial metabolism, and may arise in the alimentary tract of the higher animal. They are readily oxidised to sulphates after absorption.

(3) *Organic Sulphates.*—Esters of phenols, cresols, indoxyl, and other alcohols. They appear as detoxication products in mammalian urine. *Taurine* occurs in bile acid.

(4) *Organic Sulphides.*—Allyl sulphide, or “oil of garlic,” and ethyl sulphide occur in many *Liliaceæ*, especially onion and garlic.

(5) *Thiocyanates.*—Potassium thiocyanate, KSCN , is a frequent but obscure micro-constituent of human saliva, bile, and urine. Organic isothiocyanates, or “mustard oils,” occur as glucosides in many plants.

(6) *Thio-amino acids*, cystine, cysteine, methionine.

(7) *Thio-peptides*, glutathione.

(8) *Thiols*, thioneine.

(9) *Thiamine*, or vitamin B_1 .

(10) *Sulpholipides.*—Fat-like sulphur compounds found in brain.

(11) *Mucoitin*, or glucothionic acid, found in glycoproteins.

(12) *Chondroitin sulphate*, found in skeletal tissue of animals.

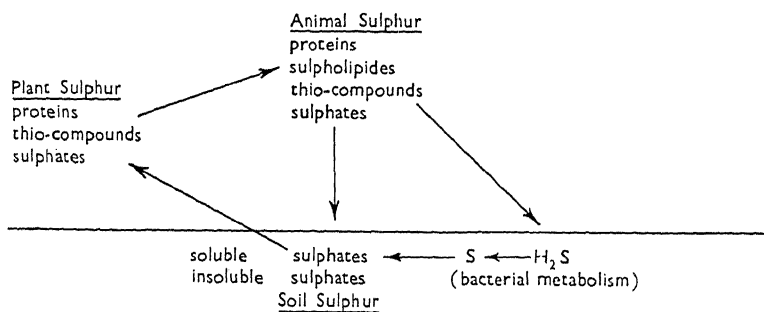
AN INTRODUCTION TO BIOCHEMISTRY

(13) *Thio-melanins*.—Skin pigments containing sulphur and amino-acid residues.

Three principal forms of urinary sulphur are recognised : (i.) inorganic sulphates of Na, K, NH_4 , Ca, and Mg ; (ii.) organic sulphate esters of phenols ; (iii.) "neutral" sulphur compounds, including thiols and salts of thio-acids.

Significance.—Sulphur assumes so many forms that it is impossible to ascribe a general significance to the element. It is necessary for protein synthesis in plants, although unassimilable as a free element. In the animal dietary, sulphur resembles phosphorus in being acidogenic. It is assimilated in the almost neutral form of thio-amino acids, obtained from proteins, and is excreted after oxidation as a salt of the strong inorganic acid, H_2SO_4 . Organic sulphates enter into the detoxication mechanism of the mammal, and are synthesised to enable the organism to eliminate alimentary and other autogenic toxins in a harmless form.

The Sulphur Cycle.—Unlike phosphorus, sulphur appears in both oxidised and reduced forms in the history of life. Completely oxidised as sulphate, it enters the plant from the soil, and is converted into partially reduced organic compounds containing the thiol group $-\text{SH}$ or the disulphide linkage $-\text{S}-\text{S}-$. From these, the completely reduced form, H_2S , is derived by bacterial degradation either in the soil or in the alimentary tract of the animal. Hydrogen sulphide is attacked by the sulphur-oxidising bacteria, with the ultimate formation of sulphate, which is available for plant absorption.



Sulphur Bacteria.—A group of organisms that obtain energy by the oxidation of H_2S into H_2SO_4 , by means of a chain of reactions. Some occur in soil, and may account for the conversion of unavailable sulphur into forms assimilable by higher plants.

The biochemistry of the sulphur bacteria has been reviewed by Bunker (1936).

Group VII : { **Manganese.**
 { *Halogens* : **Fluorine, Chlorine, Bromine, Iodine.**

Manganese.—Mn, a.n. 25 ; a.w. 54.93. Present in all plants, the values ranging from less than 1 mg. up to about 200 mg. Mn per kg. dry material. Representative figures are : wheat-bran, 100–200 ; beetroot, oats, spinach, wheat-grain, 25–100 ; edible fruits, less than 15. The maximum was found in lettuce, namely, 216.2 mg. Mn per kg. These values vary greatly with soil conditions, and the Mn content of seeds is important, since it may be the only source available for the growing plant. The Mn content of cereal grain is often equal to or greater than the Fe content. Manganese is widely but unequally distributed as a micro-constituent of higher animals, being most in liver, pancreas, lymph gland, and kidney. Recorded values range from 0.01 mg. Mn per 100 gm. fresh tissue, in muscle, up to 0.2 mg. Mn in liver. Marine animals are richer in the element, and a manganese-protein compound, *pinnaglobulin*, occurs in the blood of the mussel *Pinna squamosa*.

Sea water contains 1–10 mg. Mn per cubic metre.

Significance.—(1) *A Factor in Plant Growth.* Deficiency of manganese causes retardation of growth and chlorosis of the leaf, due to lack of chlorophyll. Slight excess of manganese is toxic, especially to barley.

(2) *An Enzyme Activator.*—Laccase, a mixture of oxidase enzymes found in many plants, is activated by manganese.

(3) *A Factor in Bone Development.*—Fresh bone contains about 0.03 to 0.1 mg. Mn per 100 gm. Lack of the element in the diet of chickens leads to *perosis*, a deformity of the tibio-metatarsal joints, and subsequent deformities (Gallup and Norris, 1938).

(4) *A Factor in Growth and Reproduction.*—Manganese acts as a growth-stimulant for young rats, and appears to be necessary for normal reproduction. It is invariably present in the reproductive organs and in the developing egg. Everson and Daniels (1930) claim that it is necessary for child nutrition, and prescribe a daily intake of 0.2–0.3 mg. Mn per kg. body-weight during the first five years.

(5) A coactant with vitamin B₁ in carbohydrate metabolism.

(6) A coactant in the synthesis of vitamin C.

Manganese in Foodstuffs.—From the surveys of Peterson (1928), Richards (1930), the richest sources are : liver, kidney, pancreas, muscle, lettuce, spinach and unmilled cereals. Wheat germ, which contains about 39 mg. Mn per 100 gm. dry material, offers an abundant supply (Sluiter, 1933 ; Von Oettingen, 1935).

Fluorine.—F, a.n. 9 ; a.w. 19. Fluorine, the most active of the

elements, is a micro-constituent of plants, reported values ranging from 6–1,000 mg. F per kg. fresh tissue. It is greatest in leaves and in skins of fruits.

The fluorine content of sea water is about 1.4 mg. per litre, and the halogen is a frequent micro-constituent of marine organisms.

Higher animals contain fluorine in skeletal tissue and viscera. Values range from 2 mg. in human brain and lung up to 15 mg. in kidney tissue, expressed as mg. F per kg. dry material. Egg yolk contains about 11 mg. per kg. of fluid. Fresh human bone contains 150–560 mg. F per kg., the maximum being in dentine. Tooth substance has been reported to contain 0.2–0.8 per cent. CaF_2 , enamel being specially rich, but Bowes and Murry (1935) claim that there is not more than 0.03 per cent. of F in both dentine and enamel. These low values may be due to dietary conditions.

Significance.—Fluorine contributes to the hardness of the skeleton, especially the teeth. There are minimal and optimal concentrations in the dietary, beyond which conditions of fluorine deficiency and fluorine poisoning, respectively, are observed. Concentrations of NaF above 0.05 per cent. in the diet of the rat cause pathological over-development of tooth enamel, and defective dentition.

Inorganic fluoride has an inhibitory action on enzymes, especially those causing activation of carbohydrates and fats.

Sharpless and McCollum (1933) find that the fluorine content of bones and teeth varies with the diet, and can be reduced almost to zero without causing a marked change in tooth structure, or affecting the general well-being of the animal, as shown by growth-rate and power of reproduction. From this, they conclude that fluorine is not an essential element in mammalian physiology. A dental defect has been traced to the use of water rich in fluorides, and mottling of the dental enamel has resulted from the consumption of drinking water containing 3.8–7.15 mg. F per litre.

The physiological effects of fluorine have been reviewed by Dyson (1927), and by McClure (1933).

Chlorine.—Cl, a.n. 17; a.w. 35.46. Chlorine has been detected in all plants, with the outstanding exception of the conifers. It is a constant micro-constituent of seeds. The chloride value of common plants is very variable, ranging from less than 0.005 per cent. of wheat ash up to 9 per cent. of lettuce ash. The halogen is an invariable constituent of the animal body, being greatest in lower marine forms and least in some fresh water species.

Percentages in fresh human tissues and fluids are: blood, 0.28–0.3; plasma, 0.35–0.38; red cells, 0.18; spinal fluid, 0.44; sweat, 0.07–0.52; urine, 0.12–0.06; lung 0.26; muscle, 0.05–0.13; milk, 0.04; gastric juice, 0.1–0.3.

The chlorine of the human body amounts to about 0.15 per cent. of the total weight. Concentration is maximal in serum and plasma exudates, gastric juice, spinal fluid, and urine ; and is minimal in brain and muscle. Urinary chloride depends on the level in the plasma, and falls almost to zero if chloride be withheld from the diet or if the plasma chloride fall below the limiting value of 0.34 per cent.

Forms of Occurrence.—Tissue chlorides can be extracted by dialysis, and it is concluded that biological chlorine is mostly ionic, although organic forms are known to exist (Nolan, 1936).

Significance.—(1) *A Transport Element.*—Chlorides of the biochemical metals are easily soluble in water and readily ionised. Chlorine thus provides a transport partner for the absorption, distribution, and excretion of other radicles.

(2) *A Factor in Hydration.*—NaCl is the principal inorganic solute of the animal, and its concentration is largely responsible for determining the amount of water held by various tissues. The effective ion appears to be Na^+ rather than Cl^- .

(3) *A Factor in Osmotic Pressure.*—NaCl is the principal electrolyte of the plasma, and largely determines the osmotic pressure level of blood.

(4) *A Buffer Agent.*—Cell membranes are more or less permeable to the Cl-ion, but relatively impermeable to the metallic ions that accompany it. Potassium is located chiefly in cells ; sodium is found chiefly in tissue fluids. The Cl-ion is able to migrate between cell and surrounding fluid in response to changes in K^+ or Na^+ concentration. This "chloride shift" occurs in blood when increase in carbonate concentration drives chlorine from plasma to red cell, or *vice-versâ*. Conversely, removal of carbon dioxide during pulmonary aeration causes chlorine to pass from the red cells to the plasma.

(5) *A Constituent of Gastric Secretion.*—The HCl of gastric juice is essential for peptic digestion. It comes from the chloride of the plasma.

(6) *A Factor in Plant Growth.*—External chloride does not appear to be necessary for plant growth as the seed carries all the necessary halogen. It is, however, necessary for seed production in the mature plant.

Bromine.—Br, a.n. 35 ; a.w. 79.92. A constant constituent of marine plants and marine animals, especially anthozoa, in which it may replace chlorine almost completely. A dibromo-indigo, the original Tyrian purple of Imperial Rome, is obtainable from the gastropods, *Murex brandaris* and *M. trunculus*. Bromine appears to be a micro-constituent of higher animals, reported values ranging

from 0.3 mg. Br per 100 gm. fresh lung tissue up to 1.5 mg. per 100 ml. blood. The occurrence is variable, and nothing is known as to the significance of the halogen.

The Br value of human blood lies between 227 and 572 γ per 100 ml., the average being 372 γ for blood, and 656 γ for urine (Conway and Flood, 1936). Impure table salt, baking powder, and the hypochlorite used in flour bleaching are common sources of bromine in the diet. (1,000 γ = 1 mg.).

Iodine.—I, a.n. 53; a.w. 126.98. A micro-constituent of all plants and animals. Seaweeds are especially rich, and form an industrial source of the element. The kelp ash of *Laminaria digitata* may yield 33–40 lb. per ton. Among land plants, the values average about 0.0005–0.01 gm. I per 100 gm. fresh material, the concentration depending more on district than on species. Watercress and other fresh-water plants have relatively higher values.

In mammalian tissue, the thyroid is noteworthy for its high content of iodine, values in man range from 9.0–40 mg. I per 100 gm. fresh tissue. Other organs have values ranging from 1 mg. in liver and kidney down to 0.01 mg. in blood. The iodine value of the thyroid depends primarily on the iodine intake, and to a lesser extent on species, age, and sex. The content is usually inversely proportional to the size of the gland, and is subnormal in all conditions of simple goitre. The blood iodine level may rise to 0.04 mg. I per 100 ml. in exophthalmic goitre, and fall to less than 0.003 in simple goitre. The recorded urinary output ranges from 0.03–0.173 mg. *per diem*. Milk contains 0.001–0.046 mg. I per 100 ml.

Forms of Occurrence.—(1) *Thyroxine* occurs as a natural amino acid in the thyroid, and is the active radicle in its internal secretion.

(2) *Iodogorgoic acid*, a di-iodide of tyrosine, occurs in spongin, the skeletal protein of sponges.

(3) Unknown micro-constituents.

Significance.—The influence of iodine on plant metabolism is uncertain. In marine invertebrates, especially sponges and corals, organic iodine forms part of the skeletal protein.

It is an essential micro-constituent of the higher animal, the minimal daily requirement for man being about 0.05 mg. I_2 . Elaborated into thyroxine by the thyroid apparatus, iodine controls the basal metabolic rate of animal metabolism. The distribution of iodine has been surveyed by McClendon (1927), Orr and Leitch (1929), and Orr (1931).

Group VIII : Iron, Cobalt, Nickel.

Iron.—Fe, a.n. 26; a.w. 55.84.

Iron is the fourth element in order of abundance in the earth's

crust, and is a micro-constituent of all living tissues. Iron is universal in the green parts of plants, the values ranging from about 5–10 mg. per 100 gm. of dry material. It also occurs, but to a much lesser extent, in roots, tubers, and in plants free from chlorophyll. Vegetable foodstuffs in order of decreasing value are: dried legumins, green leafy vegetables, dried fruits, nuts, cereals, green legumins, roots, tubers, fruits.

Iron is universal in animals, its micro-concentration being 0.01–0.1 mg. per gm. of cell substance. In addition, it is an invariable secondary element in all the red-blooded animals. The iron content of mammalian blood is approximately 50 mg. per 100 ml., mostly in the form of *hæmoglobin*, which contains 0.3–0.5 per cent. Fe.

The human adult contains about 0.01 per cent., or 3–5 gm. Fe., in terms of total body weight, of which 2–2.5 gm. circulates as blood pigment.

Representative iron percentages of fresh animal tissues and fluids are: brain, 0.002; hair, 0.08; kidney, 0.005; liver, 0.008; lung, 0.01; muscle, 0.004; pancreas, 0.006; spleen, 0.009; human milk, 0.0004; cow's milk, 0.0002–0.001; hen's egg, 0.0025; hen's egg yolk, 0.007.

For a natural food material, milk is remarkably deficient in iron, and the young animal relies largely on the metal stored in its liver before birth.

Iron is absorbed from the intestine as ferrous ions, Fe^{++} , liberated by gastric HCl acting on the food materials. If absorption be delayed, ferrous iron is oxidised to ferric iron in the alkaline regions of the small intestine, or may combine with sulphides and phosphates to form insoluble salts. Under these conditions, iron is non-available, and is excreted by the intestine. Iron in the organic form of hæm is not readily released by gastric digestion. McCance found that 15–18 mg. of iron are retained when 50 mg. are given daily as sulphate or chloride to a subject in a state of iron equilibrium, but none is retained when the 50 mg. are given in the form of hæm. For this reason, muscle and dried blood are poor sources of nutritional iron. Assimilated iron is not returned to the intestine, which, according to McCance, "has no power of regulating by excretion the amount of iron in the body" (1938).

Forms of Occurrence.—Tissue iron occurs as a porphyrin derivative, *hæm*, from which are derived the cytochromes and other hæmochromes present in all aerobic cells. Iron also occurs as the mobile respiratory pigments of blood, *chlorocruorin* and *hæmoerythrin* in lower animals, and *hæmoglobin* in higher animals. The enzymes *catalase*, *peroxidase*, and *histaminase* are proteins united to hæm.

Significance.—The biological function of iron is the transference of oxygen from the environment to the oxidisable metabolites in the

tissues. In higher animals, this is effected by chains of reacting compounds, which include hæmoglobin and cytochrome *c*.

In the green plant, iron appears as a constituent of the chloroplasts. It is not part of the chlorophyll molecule, but is necessary for chlorophyll formation. When iron is withheld from plants, local chlorosis develops, and can be cured by application of iron salts to the soil.

In the higher animal, iron is stored chiefly in liver and spleen, but only a small part of this is useful for hæmoglobin manufacture. In the treatment of anæmia, the need for large doses of iron depends partly on the low assimilability of the metal, but chiefly on account of the activity of the storage mechanism, "which must be flooded with iron if any is to overflow and be available for hæmoglobin synthesis."

In some species (human subjects and rodents) copper is an essential supplement of iron in hæmoglobin manufacture (Hutchison, 1938).

A Factor in Plant Growth.—The response of plants to iron depends both on the nature of the salt supplied and the nature of the soil. A slight deficiency of iron can diminish greatly the yield of a crop without causing much change in appearance; an acute deficiency alters the colour of the leaves owing to defective chlorophyll formation.

Effect of other Metals on Iron Assimilation.—Iron metabolism in higher animals appears to be catalysed by traces of copper. Similar effects have been claimed for related metals, including manganese, nickel, and cobalt, but the results of different investigations are at variance.

Oxidation Inhibitors.—Cyanides, hydrogen sulphide, and carbon monoxide react with hæmatin compounds containing ferrous iron, and each of these reagents will inhibit the respiration of all tissues. This led Warburg to conclude that an iron catalyst is concerned in cellular respiration, and his conclusions have been confirmed by the discovery of the cytochromes.

Iron Bacteria.—These higher bacteria inhabit ferruginous waters, and are characterised by a cell membrane impregnated with ferric hydroxide, which results in the formation of fluffy, brownish streamers on stones or plant stems under water. It was at one time believed that these organisms obtained their energy by the oxidation of ferrous salts to ferric, but it is now known that their metabolism is orthodox. The colour is due to local precipitation of iron salts, and is not a unique metabolic process. During their life period, iron bacteria cause the accumulation of ferric hydroxide in natural waters containing soluble iron salts.

The Iron Cycle.—Iron occurs on the surface of the earth chiefly in union with oxygen as ferrous oxide, FeO , and ferric oxide, Fe_2O_3 . The former, FeO , is a strong base, able to form neutral salts with most acids. The latter, Fe_2O_3 , is a weak base, unable to fix CO_2 as a stable carbonate. Ferrous silicates of rocks and soil are decomposed by atmospheric CO_2 , and yield ferrous carbonate. This is oxidised to ferric oxide, and the liberated CO_2 is returned to the atmosphere. Ferric oxide can act as an oxygen donator to the organic matter in the soil, and thus is reduced to ferrous compounds. These are redistributed by the soil water, and reoxidised to ferric oxide. Iron is therefore a continuous oxidising agent, preventing the retention of carbon in soil, and enabling it to return to the atmosphere as CO_2 .

Cobalt.—Co, a.n. 27; a.w. 58.97.

Nickel.—Ni, a.n. 28; a.w. 58.68.

These closely related metals are widely distributed micro-constituents of plants and animals, reported values ranging from 0.002 to 2 parts per million fresh tissue (Bertrand). In plants, Ni occurs chiefly in the leaf, ranging from 1.51 mg. per kg. dry material in lettuce to 3.3 mg. in cabbage. Both metals were found in many of the marine organisms examined by Fox and Ramage, although neither Co nor Ni has yet been detected in sea water. Bertrand claims that both Co and Ni are normal constituents of the pancreas, and necessary for its functioning. Cobalt has been shown to be effective in raising the blood volume and red cell concentration in rats, and to be a specific cure for the disease, enzootic marasmus, or bush sickness, found in sheep and cattle in some parts of Australia and New Zealand, and now ascribed to deficiency of Co in the soil and herbage (Underwood, 1937). Cobalt is an activator of the enzyme arginase, and thus may be concerned in the mechanism for urea manufacture in the liver. Neither metal has yet been shown to be an essential factor in the human dietary. Nickel is present in tea, and cobalt is a contaminant of many therapeutic preparations of iron.

General Significance of the Biochemical Elements.—When the composition of a higher organism is compared with that of its environment several features of distinction are seen. Thus, silicon and aluminium, that together as the aluminium silicate of clay make up a third of the earth's crust, are found only as micro-constituents in man, and give little indication of a dusty ancestry. On the other hand, hydrogen, carbon, and nitrogen, the building stones of life, occur only as secondary constituents of the environment.

The suitability of the biological elements would appear to be set beyond discussion by the mere fact of their presence in the organism.

Underlying this, however, is the deeper question as to whether the determinants of organisation are, in turn, determined by the properties of the elements, or whether there is a process of chemical selection at work gradually altering and improving the framework of life.

RELATIVE COMPOSITION OF THE HUMAN ORGANISM AND THE ENVIRONMENT

ORGANISM Percentage composition		ENVIRONMENT Percentage composition	
Oxygen	63.03	46.68	Oxygen
Carbon	20.20	27.60	Silicon
Hydrogen	9.90	8.05	Aluminium
Nitrogen	2.50	5.03	Iron
Calcium	2.45	3.63	Calcium
Phosphorus	1.1	2.72	Sodium
Chlorine	0.16	2.56	Potassium
Fluorine	0.14	2.07	Magnesium
Sulphur	0.14	0.7	Titanium
Potassium	0.11	0.15	Phosphorus
Sodium	0.10	0.15	Carbon
Magnesium	0.07	0.11	Hydrogen
Iron	0.01	0.1	Manganese
Micro-constituents	< 0.01	0.09	Sulphur
		0.09	Chlorine
		< 0.09	Micro-constituents

Rough data, such as those tabulated above, show the power of the living organism to select the necessary biological elements irrespective of their relative concentrations, and its inability or reluctance to utilise some of those most readily available.

Only eight elements are present in the earth's crust in concentrations of more than 1 per cent. They are : O, Si, Al, Fe, Ca, Na, K and Mg, and together make up 98 per cent. of the surface. About 1.1 per cent. is made up of Ti, P, Ca and H (as H_2O). The remaining 80 elements together constitute only 0.5 per cent. of the crust.

A 70 kg. human body contains approximately: oxygen, 46 kg.; carbon, 12 kg.; hydrogen, 7.2 kg.; nitrogen, 1.7 kg.; calcium, 1.1 kg.; phosphorus, 630 gm.; chlorine, 112 gm.; fluorine, 60–100 gm.; sulphur, 110 gm.; potassium, 90–100 gm.; sodium, 95 gm.; magnesium, 60 gm.; iron, 4–5 gm. Variations depend on the state of nutrition, the relative fat content and the size of the skeleton.

The availability of the biological elements fixes the limits to the expansion of life. This was recognised by Liebig in 1843, and expressed as his familiar “law of the minimum.” The yield of any crop is determined by the nutrient present in least amount.

L. J. Henderson has developed the concept of environmental fitness, and has shown that the unique properties of the primary elements, carbon, hydrogen, and oxygen, endow their compounds with the maximal fitness for the manifestation of life. This concept has not yet been extended to the other elements, although it is interesting to speculate on the biological importance of magnesium and iron. Without the former there would be no chlorophyll, without the latter there would be no hæmoglobin.

Marett (1936) believes that the mineral deficiencies of the soil operate in determining race and destiny.

The Geochemical Changes due to Life.—Life, for all its abundance, does not occupy a large volume of the earth's crust, but the effects of its precarious tenancy are shown by its architectural achievements, the chalk cliffs and coral islands, which are monuments of many thousand years of biological industry. In 1875, Suess introduced the term *biosphere* to denote the portion of the earth occupied by life, and, in 1918, Vernadsky began an investigation into the extent to which the maintenance of this biosphere had influenced the surface history of the earth. The Biogeochemical Laboratory of the Russian Academy of Sciences was created in 1928, and since then important surveys have been published by Vernadsky and by Vinogradov and his colleagues, in support of the conclusion that the chemical composition of an organism is characteristic of the species, and that the composition of living matter in general can be regarded as a function of the atomic weights of the elements, every sixth of which, in periodic order, appears to have a special importance in life.

Geologically ancient types, such as bacteria and foraminifera, are able to concentrate a much wider range of elements than those accumulated by the highest modern types, such as birds and mammals.

GENERAL REFERENCES

- BUNGE, G. (1902), "Physiological and Pathological Chemistry." London.
- BUNKER, H. J. (1936), "Physiology and Biochemistry of the Sulphur Bacteria." *Sci. Ind. Res. Sp. Rep.*, 3. London.
- FEARON, W. R. (1933), "A Classification of the Biological Elements." *Sci. Proc. Roy. Dublin Soc.*, 20, 531.
- FEARON, W. R. (1936), "Nutritional Factors in Disease." London: Heinemann.
- FOX, H. M., and H. RAMAGE (1931), "A Spectrographic Analysis of Animal Tissue." *Proc. Roy. Soc.*, B, 108, 157.
- HENDERSON, L. J. (1924), "The Fitness of the Environment." London.
- KING, E. J., and T. H. BELT (1938), "Physiological and Pathological Aspects of Silica." *Physiol. Rev.*, 18, 329.
- LEWIS, H. B. (1924), "Sulfur Metabolism." *Physiol. Rev.*, 4, 394.
- LOTKA, A. J. (1925), "Physical Biology." Baltimore.
- MCCANCE, R. A. (1936, 1), "Medical Problems in Mineral Metabolism." *Lancet*, 230, 643, 704, 764, 823.
- MCCANCE, R. A. (1936, 2), "Experimental Sodium Chloride Deficiency." *Proc. Roy. Soc.*, B, 814, 245.
- MCCLURE, F. J. (1933), "A Review of Fluorine and its Physiological Effects." *Physiol. Rev.*, 13, 277.
- MARETT, J. R. (1936), "Race, Sex and Environment." London.
- MINOT, A. S. (1938), "Physiological Effects of Small Amounts of Lead." *Physiol. Rev.*, 18, 554.
- ORR, J. B., and I. LEITCH (1929), "Iodine in Nutrition." *Med. Res. Council Rep.*, 123.
- OSBORN, H. F. (1918), "The Origin and Nature of Life." London.
- RAMAGE, H. (1936), "Biological Distribution of Metals." *Nature*, 137, 67; 138, 762.
- SHELDON, J. H. (1934), "Mineral Basis of Life." *Brit. Med. J.*, i., 47.
- STEWART, C. P., and G. H. PERCIVAL (1928), "Calcium Metabolism." *Physiol. Rev.*, 8, 283.
- UVAROV, B. P. (1934), "Geochemistry of Living Matter." *Nature*, 134, 11.
- VERNADSKY, V. I. (1929), "La biosphère." Paris: Masson.
- VINOGRADOV, A. P. (1935), "Geochemistry and Biochemistry." *Current Sci.*, 4, 4.
- VINOGRADOV, A. P. (1935-37), "The Biological Elements," (in Russian). *Trans. Biogeochemical Lab.*, No. 3, 4. Leningrad-Moscow.
- WEBB, D. A. (1937), "Spectrographic Analysis of Marine Invertebrates." *Sci. Proc. Roy. Dublin Soc.*, 21, 505.
- WEBB, D. A., and W. R. FEARON (1937), "Studies in the Ultimate Composition of Biological Material." *Sci. Proc. Roy. Dublin Soc.*, 21, 487.

CHAPTER III

INORGANIC COMPOUNDS

"One may not doubt that, somehow, Good
Shall come of Water and of Mud ;
And, sure, the reverent eye must see
A Purpose in Liquidity."

RUPERT BROOKE.

WITH the exception of oxygen, and traces of dissolved hydrogen and nitrogen, elements do not occur free in living organisms, but are present as inorganic and organic compounds. As a class, inorganic compounds are non-combustible, and, with the exception of the carbonates, do not contain carbon. Bio-organic compounds, on the other hand, are combustible, and all contain carbon and hydrogen.

Animal and vegetable ash after complete incineration is composed entirely of inorganic salts and oxides, and such compounds were the first investigated. Subsequently, analyses were made of tissue extracts and secretions after separation of organic compounds by dialysis or precipitation. This method gives more significant information, since it deals with salts and ions actually present during life, but, even so, there is no doubt that many inorganic radicles occur united to organic compounds, both in hard tissues, such as bone, and in soft tissues, such as muscle.

Inorganic Biochemical Compounds.—The most important are : water, carbon dioxide, the carbonates, carbamates, silicates, sulphates, phosphates, fluorides, and chlorides of the biochemical metals ; the nitrogen derivatives, ammonia, nitrous and nitric acid.

Water.—Water comes first in quantitative importance among biochemical compounds, and is the solvent in all vital reactions. The average water content of land animals is about 60–70 per cent. of the total weight. It may be as low as 10 per cent. in some insects, and in latent forms of life, such as seeds and spores ; and may exceed 95 per cent. in lower forms of marine life, such as jelly fish.

The water content of mammalian tissue is roughly proportional to physiological activity, and inversely proportional to fat-content. It decreases with age. The human embryo at the end of the third

month contains about 94 per cent. At birth, this has fallen to 67, and in adult life it is fairly constant between 60-63 per cent.

Percentage Water Content of Human Tissues

Tissue.	New-born.	Adult.
Blood . .	85.0	77.9-83.0
Muscle . .	81.8	73.0-75.7
Brain . .	89.3	75.0-82.0
Bone . .	32.3	22.0-34.0
Lung . .	82.6	78.0-79.0
Liver . .	80.5	68.3-79.8
Kidney . .	85.7	77.0-83.7

The 40-45 kg. of water incorporated in the human adult are located chiefly in the muscles. Adolph (1933) computes the total turnover of water between tissues, blood and alimentary tract, to range from 4.75 to 17.6 litres *per diem*, involving a loss of 1.05-7.8 litres, through the channels of the kidneys, colon, lungs and skin, the average daily output being 3.4 litres. This must be made good by the water obtained from the diet.

Close (1934) has classified the various regions of the body according to water content: (a) Extracellular body fluids, plasma, lymph, cerebro-spinal and synovial fluids; (b) cellular tissues rich in nuclei, glands, muscles, grey nervous tissue; (c) supporting tissues poor in nuclei, connective tissue, cartilage, bone, white nervous tissue.

Characteristic.	TISSUE GROUP.		
	(a) Body Fluids.	(b) Nuclear Tissues.	(c) Aneuclear Tissues.
Water percentage .	90-99	70-85	70
Metabolic activity .	nil	high	low
H-ion reaction .	alkaline pH 7.3-7.5	acid pH < 7.0	midway
Na ⁺ content .	high	low	midway
K ⁺ content .	low	high	midway
Protein content .	low	midway	high
Cl ⁻ content .	high	low	midway
Cl ⁻ : H ₂ O ratio .	increases with increase in H ₂ O	increases with increase in H ₂ O	decreases with increase in H ₂ O

Free Water and Bound Water.—The organism is a cellular, colloidal structure, and contains water in two chief forms: free

water, or *water of solution*; bound water, or *water of constitution*, combined with tissue colloids. Free water circulates as a solvent, and varies according to diet, metabolism, and excretory activity. Bound water is an integral part of the tissues, and reaches a constant value in mature life.

Estimation of Free Water.—(a) When a solute (e.g., sucrose or urea) is added to a given volume of water it causes a corresponding fall in the vapour pressure of the mixture. When the same solute is added to a tissue containing the same total volume of water the depression of the vapour pressure is never as great as it is with the pure solvent. The difference is due to the fact that some of the water is "bound," and is not available for solution. Consequently, the "free" water fraction may be measured by adding a known amount of a suitable solute, and comparing the resulting depression of vapour pressure with that caused by adding the same amount of solute to an isotonic saline.

Hill (1930) defines the "free" water fraction as the weight of water in 1 gm. of fluid or tissue which can dissolve solutes with a normal depression of vapour pressure. In 1 gm. frog muscle, the total water is 0.80, and the "free" water is 0.77 gm., showing that very little of the water in muscle tissue is "bound."

Functions of Water.—(1) *Structure.*—Water determines the bulk of tissues and organs, and renders them plastic while incompressible.

(2) *Nutrition.*—All the food materials of plants and animals are assimilated in aqueous solution. Water, in addition, is the chief quantitative constituent of the dietary.

(3) *Anabolism.*—In green plants, water is utilised in the synthesis of bio-organic compounds, including carbohydrates.

(4) *Catabolism.*—Water is the major end-product of plant and animal metabolism.

(5) *Transport.*—The food materials, the internal secretions, and the waste products of the organism are distributed in aqueous solution.

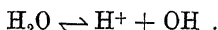
(6) *Temperature Stabilisation.*—The high specific heat of water enables the organism to store heat and maintain a uniform temperature. The high latent heat of water renders it very efficient as a cooling agent when evaporating from the surface of the organism.

(7) *Solvent.*—Water is a powerful ionising solvent, and ionisation is a preliminary stage in almost all the reactions taking place within the organism.

Ionic Systems.—All aqueous solutions can be divided into (a) electrolytes, and (b) non-electrolytes. Electrolytes are characterised by their ability to conduct electricity, and by the fact that many of their physical properties, such as osmotic pressure and boiling point, are in excess of those calculated from the molecular weight of the solute, showing that solution has been attended by an increase in

the number of particles dissolved. Non-electrolytes have minimal electric conductivity, and their properties indicate that no increase has taken place in the units of solute. The difference is ascribed to the presence of *ions* in the electrolytes. These are derived spontaneously from the solutes, and their concentration determines the conductivity of the solution. Solutions containing no ions have zero conductivity.

Water is the most effective of the ionising solvents, and even when pure has a low conductivity of its own, indicating a slight degree of ionisation :



The H^+ , or proton, being highly reactive, combines with water molecule to form the *hydroxonium* ion, H_3O^+ , which is also written $\text{H}_2\text{O}:\text{H}^+$, to show that the proton is in semi-polar union with H_2O , and can be removed easily by a proton acceptor.

Salts, even of weak acids or bases, are almost completely ionised in aqueous solution, consequently all the tissues and fluids of the organism contain a mixture of various ions. Some of these mixtures form natural systems of considerable importance in life. These include :

(1) *The hydrogen ion buffer system*, whereby the acidity and alkalinity of the tissues are kept within proper working range.

(2) *The oxidation-reduction systems* participating in tissue metabolism.

(3) Various *metallo-ionic systems* influencing tissue water content, and physiological sensitivity.

The H-ion Concentration of Living Tissues.—Since all forms of life contain water, and all aqueous solutions contain H-ions and OH-ions, it follows that these are invariable constituents of living tissues. In pure water, or in other neutral solutions, the H-ion concentration, usually written $[\text{H}^+]$, exactly equals the OH-ion concentration, or $[\text{OH}^-]$. In acid solutions, $[\text{H}^+]$ is greater than $[\text{OH}^-]$. In alkaline solutions, $[\text{OH}^-]$ is greater than $[\text{H}^+]$. However they may vary individually, the product $[\text{H}^+] \times [\text{OH}^-]$ has a constant value for a given temperature. That is to say, neither $[\text{H}^+]$ nor $[\text{OH}^-]$ can ever equal zero. The strongest acid solution obtainable contains OH-ions; and the strongest alkaline solution has H-ions.

The activities of life are essentially acidogenic in that carbon dioxide is an end-product of the oxidation of all organic compounds. In consequence, the H-ion concentration of the tissues and tissue fluids is continually tending to increase, and must be neutralised by appropriate bases if the chemical equilibrium of the organism is to be preserved.

Acids are substances capable of liberating H-ions.

Bases are substances capable of combining with H-ions.

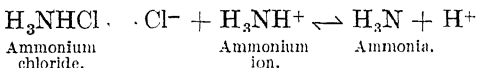
In the anhydrous state, acids, such as HCl gas, pure HNO₃ and pure H₂SO₄, contain no free H-ions. On addition of water, or other ionising solvents, ionisation takes place to an extent determined by the *strength* of the acid. Strong acids are almost completely ionised; weak acids, except in great dilution, are only partially ionised. The degree of ionisation is expressed either as (i.) the percentage of acid present in the form of free ions, or as (ii.) the *dissociation constant* of the acid, K, which is the product of the concentrations of both component ions, [H] × [A], divided by the concentration of the unionised molecules of acid, [HA].

Acid.	Ions Liberated.	Percentage Ionisation in 0.1 M Solution.	Dissociation Constant (K) $= \frac{[H] \times [A]}{[HA]}$
Hydrochloric	H ⁺ + Cl ⁻	> 90	> 10 ⁶
Sulphuric	H ⁺ + HSO ₄ ⁻	> 90	> 10 ⁶
Sulphurous	H ⁺ + HSO ₃ ⁻	34	1.7 × 10 ⁻²
Phosphoric	H ⁺ + H ₂ PO ₄ ⁻	24	7.6 × 10 ⁻³
Acetic	H ⁺ + CH ₃ .COO ⁻	1.33	1.8 × 10 ⁻⁵
Carbonic	H ⁺ + HCO ₃ ⁻	0.18	3.1 × 10 ⁻⁷
Hydrogen sulphide	H ⁺ + HS ⁻	0.09	8 × 10 ⁻⁸
Boric	H ⁺ + H ₂ BO ₃ ⁻	0.01	6 × 10 ⁻¹⁰

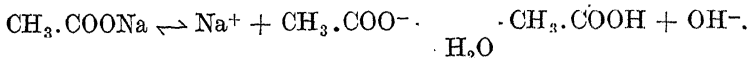
It will be seen from this table that dihydro- and trihydro-acids only release one of their acidic hydrogens in 0.1 M concentrations. By greatly increasing the dilution of the acid, all the acidic hydrogen is ionised, whatever be the nature of the acid. Acid salts, such as NaH₂PO₄, NaHSO₄ and NaHCO₃, also liberate H-ions, but to a much lesser degree than the parent acid. The ionisation of very strong acids, such as HCl, can only be computed approximately in concentrated solutions, owing to ionic attraction effects.

Bases undergo a corresponding type of ionisation in aqueous solution, and liberate OH-ions, which are able to combine with H-ions to form water. The strength of a base is due to the number of OH-ions liberated at a given dilution. Thus, NaOH and KOH are almost completely ionised in 0.1 M concentrations, while 0.1 M NH₄OH, a weak base, is ionised only to the extent of 0.4 per cent.

Salts are compounds formed by the union of acidic and basic radicles. When dissolved in water they are ionised into their constituent positive and negative ions. If the positive ion is derived from a weak base, such as ammonia, it tends to lose a proton, and so increase the H-ion concentration of the solution.



Conversely, the salt of a weak acid and a strong base forms an alkaline solution, owing to the tendency of the weak acidic ion to abstract H-ions from the water, and so increase the OH-ion concentration of the mixture.



Neutral solutions are those in which the H-ion concentration exactly equals the OH-ion concentration. Pure water is taken as the absolute standard of neutrality. At a temperature of 22° C., a litre of water contains 10^{-7} gram-ions of H^+ and 10^{-7} gram-ions of OH^- . Otherwise expressed, there is 1 gm. of ionised hydrogen in 10^7 , or 10,000,000, litres of water at 22° C.

For brevity, these concentrations in gram-ions per litre are written C_{H} and C_{OH} . The C_{H} of pure water, or any neutral solution at 22° C. is 10^{-7} . Any solution with a C_{H} greater than 10^{-7} is acid; and any solution with a C_{H} less than this value is alkaline. The product $C_{\text{H}} \times C_{\text{OH}}$ always equals 10^{-14} , so either acidity or alkalinity can be expressed in terms of C_{H} , which is written $[\text{H}^+]$.

$$[\text{H}^+] \times [\text{OH}^-] = k \text{ (a constant),}$$

$$\therefore [\text{H}^+] = \frac{k}{[\text{OH}^-]}, \text{ and } [\text{OH}^-] = \frac{k}{[\text{H}^+]}$$

Electrometric Determination of C_{H} . The reaction, or H-ion value of a solution can be measured by three different types of instrument: (i.) the *hydrogen electrode*, (ii.) the *quinhydrone electrode*, and (iii.) the "glass" electrode. In the first method, a platinum electrode with a layer of adsorbed hydrogen is immersed in a solution of known C_{H} , a similar electrode being immersed in the solution of unknown C_{H} . The two solutions are connected by a narrow tube containing an electrolyte, KCl, kept from diffusing by means of gelatin or agar (Fig. 1).

H-ions tend to leave each electrode and pass into solution, the extent of their migration being opposed by the concentration of the H-ions already present in each solution. If the two solutions have the same C_{H} , the same number of H-ions will leave each electrode, and if the electrodes are connected with a suitable potentiometer, no difference in electropotential can be found to exist between them.

If, however, one solution has a lower C_{H} than the other, that solution will allow more H-ions to migrate from its electrode, which in consequence will become negatively charged when compared with the other electrode.

Knowing the potential difference, e , between the electrodes, and the value of C_{H} for one solution, the H-ion concentration in the

other solution can be found from the formula, in which C_H^1 is the H-ion concentration in the more acid solution :

$$e = 0.0577 (\log_{10} C_H^1 - \log_{10} C_H^2) \text{ volts.}$$

It is now customary to employ the symbol pH instead of the more cumbersome $-\log_{10} C_H$, when the formula becomes

$$e = 0.0577 (pH^1 - pH^2), \text{ or } pH^1 = pH^2 + \frac{e}{0.0577}.$$

In the quinhydrone method, the platinum electrodes are not kept coated with hydrogen, but, instead, a little quinhydrone ($C_6H_4O_2 \cdot C_6H_6O_2$) is added to each solution. Quinhydrone is a compound, the oxidation-reduction state of which depends on the C_H of its solvent, and this in turn affects the potential of the immersed electrode. The readings and calculation are similar to those of the hydrogen electrode method, and the procedure is more convenient, but has the serious limitation of being inapplicable to alkaline solutions, which destroy the quinhydrone.

In the "glass" electrode method, the two solutions are separated by means of a thin glass membrane, and the potential difference of the electrodes is measured by means of a specially sensitive potentiometer.

The pH Notation.—The reaction of any solution or tissue can be expressed as 1×10^{-n} gm. H^+ per litre. In practice, this is abbreviated, and written in terms of the exponent n , which is called the pH of the solution. Otherwise defined, pH is equal to the logarithm of the volume in litres of solution that contains 1 gm. H^+ , or, more briefly,

$$pH = -\log_{10} C_H, \text{ and } C_H = 10^{-pH}.$$

True neutrality at 22° C. is pH 7, and 7 is the logarithm of 10,000,000, the volume of water in litres that contains 1 gm. H^+ . The more acid the solution the smaller will be the volume containing 1 gm. H^+ , and the lower the pH. Hence, *all acidic solutions have a pH value below 7, and all alkaline solutions have a pH value above 7.*

To appreciate the pH notation it is important to remember that pH *decreases* with *increase* in acidity, and, furthermore, since pH is logarithmic, each change in a pH unit represents a ten-fold increase or decrease in C_H .

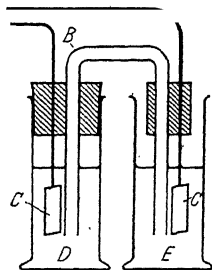
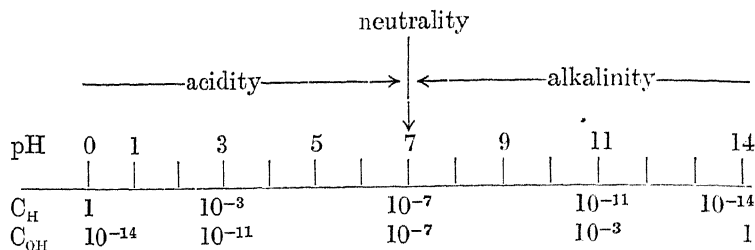


FIG. 1.—Cell composed of two quinhydrone electrodes. A—leads to apparatus for measuring E.M.F. B—siphon filled with KCl in agar. C—platinum electrodes. D—solution of known pH . E—solution of unknown pH .



The pH Scale.—H-ion concentration, or *actual acidity*, of a solution must be distinguished clearly from *available* or *titration acidity*, which is measured by finding the amount of alkali required to neutralise the solution. The pH value depends on the quality as well as the quantity of the acid; the titration value depends only on the quantity of total available acid. That is to say, pH is a measure of the number of acidic ions, while titration acidity is a measure of the number of acid molecules present, irrespective of whether they are ionised or not.

Decinormal HCl is ionised to the extent of about 80 per cent., and has a pH of about 1.09; decinormal acetic acid is only ionised to the extent of about 0.4 per cent. and has a pH of about 2.85.

Hence, N/10 HCl is a corrosive poison, whereas N/10 CH_3COOH resembles the weak vinegar that it so often impersonates.

Similar volumes of decinormal hydrochloric and acetic acid have the same neutralising power towards alkalis, although one is a very strong acid and the other is a very weak acid.

Biologically, the actual reaction or pH of a solution is more significant than the neutralising power in terms of a standard acid or alkali.

Actual Acidity

Depends only on the concentration of free H-ions present.

Estimated electrometrically or colorimetrically.

Expressed in terms of $[\text{H}^+]$ or of pH.

Available Acidity

Depends on the number of acid molecules present, irrespective of ionisation.

Estimated by titration with standard alkali.

Expressed in terms of alkali required to neutralise a given volume.

Neutralisation Curves.—When a strong acid is titrated with a strong base, and the accompanying changes in pH are measured, a characteristic curve is obtained showing an enormous alteration in

pH near the neutral point. A single drop (0.05 ml.) of decinormal acid or alkali is sufficient to shift the pH value down or up by about six units. When a weak acid is titrated with a weak base, a different type of curve is obtained, in which the equilibrium point is reached gradually, and there are no sudden changes in pH.

Buffer Systems.—Solutions of weak acids and their salts, or weak bases and their salts, constitute buffer systems, so called because they are able to neutralise alkalis or acids without undergoing marked change in pH. For example, sodium acetate can depress the acidity of HCl, and is used for this purpose in the phenylhydrazine test for sugars (p. 115).

The H-ion concentration of a typical buffer system can be found from the equation,

$$C_H = K \frac{[\text{free acid}]}{[\text{salt}]},$$

where K is the dissociation constant of the acid.

In a half-neutralised solution, the concentrations of free acid and salt are equal, and C_H becomes equal to K. Hence the dissociation constant of an acid (or a base) can be found by determining the C_H or pH of a half-neutralised solution. The pH of such a solution is termed the pK, or dissociation exponent of the acid (or the base, when an alkali is half-neutralised). Furthermore, the C_H of a half-neutralised solution is almost independent of dilution, since it is determined by the dissociation constant which, unlike the degree of dissociation, does not alter with dilution. Thus, a solution containing equivalent amounts of acetic acid and acetate ions has a pH of 4.75, and a solution containing equivalent amounts of ammonium ions and ammonia molecules has a pH of 9.48, (Fig. 2).

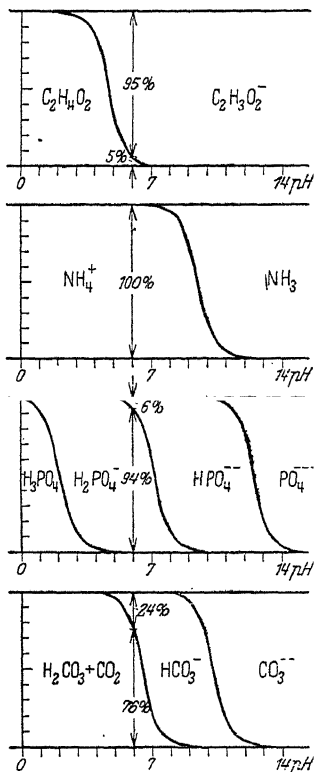
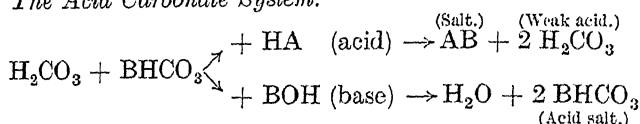


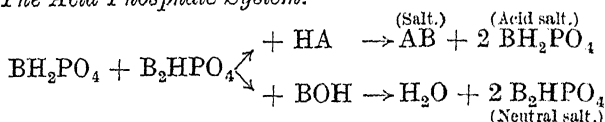
FIG. 2.—State of acids and bases at different pH values for acetic acid, ammonia, phosphoric acid and carbonic acid.

Prominent among these buffer systems of the animal body are the following :—

(1) *The Acid Carbonate System.*



(2) *The Acid Phosphate System.*

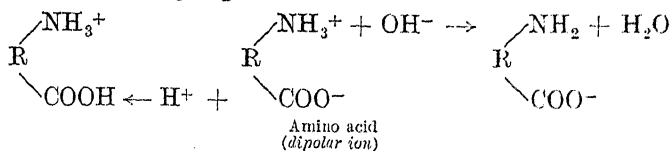


(3) *The Protein System.*

Of these systems, protein buffers are probably of greatest importance in tissues and the blood ; the carbonates come next and constitute the bulk of the alkaline reserve of the animal. Phosphate and phosphagen systems operate during muscular contraction. In blood plasma the normal ratio of $\text{BHCO}_3 : \text{H}_2\text{CO}_3$ is 20 : 1.

At the reaction of blood, pH 7·4, this acid carbonate system is not exerting its maximum effect, but as the acidity of the blood increases the buffer action becomes more powerful, and thus constitutes an important alkaline reserve of the organism. The acid phosphate system is more effective at pH 7·4, but the low concentration of phosphates in blood renders the system almost insignificant.

Proteins and amino acids, the structural units of the protein molecule, owe their buffering power to the fact that they exist in solution as dipolar or *zwitter*-ions, each of which carries positively charged $-\text{NH}_3^+$ groups and negatively charged $-\text{COO}^-$ groups. The modern concept of acidity and alkalinity, largely due to the work of the Danish school of physical chemistry, has shown that the $-\text{NH}_3^+$ group acts as an acid by releasing its proton to form H_2O by union with the OH^- ion provided by a base ; while the $-\text{COO}^-$ group acts as a base by combining with a proton, so as to form the unionised carboxyl group $-\text{COOH}$.



H-ion Indicators.—These are reagents that change colour sharply at particular changes in H-ion concentration. A series has been prepared covering the entire range from extreme acidity, pH 0·1, to extreme alkalinity, pH 13·5 [cf. Appendix].

The H-ion Concentration of the Environment.—(1) *The Ocean.*—Sea water is slightly alkaline, the usual range being pH 7.75–pH 8.25. It is increased by the photosynthetic activity of marine organisms, and Atkins has recorded a value as high as pH 9.7 in the waters of Plymouth Sound.

(2) *The Soil.* Fertile soil has a range of about pH 3–pH 10. Values as acid as pH 1.7 have been found in America, and as alkaline as pH 11 in Egypt. Chalk soil can never be more alkaline than pH 8.4.

The pH of soil is of primary importance in determining the growth and distribution of plants. Sugar beet, for example, grows best between pH 8 and pH 6; flax grows best between pH 6 and pH 4.

The H-ion Concentration of the Organism.—As a rule, plant tissues and fluids are acid, the average value being about pH 5.2; whereas animal tissues at rest, and tissue fluids, tend to be stabilised at a slight degree of alkalinity, about pH 7–pH 7.5. Metabolism is generally acidogenic in character owing to the liberation directly or indirectly of carbonic acid. Gastric juice is remarkable in having an acidity nearly as great as N/10 hydrochloric acid.

Average pH Values of Human Tissue Fluids and Secretions

Blood	7.35–7.5	Gastric juice	1.7–2.0
Urine	5.0–7.0	Pancreatic juice	7.8
Milk	6.5–7.0	Intestinal juice	7.7

In man, the range of the tissue fluids, or “internal environment,” is stabilised within the limits pH 7.0 and pH 7.8. The acid limit is marked by the onset of acidosis and coma; the alkaline limit is marked by the onset of tetany.

Control of H-ion Concentration in Man.—Four main factors operate :—

- (1) The buffer systems of fluids and tissues.
- (2) The respiratory excretion of CO_2 by the lungs.
- (3) The compensatory excretion of acids or bases by the kidney.
- (4) The metabolic formation of ammonia in the kidney and, probably, elsewhere.

Carbon dioxide and carbonic acid.—Oxidised carbon circulates in five forms within the organism : the anhydride CO_2 , as a gas and as a solute; carbonic acid $(\text{HO})_2\text{CO}$; acid carbonates, $\text{BO} \cdot \text{C}(\text{O})\text{OH}$; carbamates, $\text{BO} \cdot \text{C}(\text{NH})\text{OH}$; and is immobilised in the skeleton as normal carbonates, XCO_3 , where B is a monovalent base and X is a divalent metal.

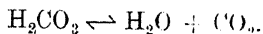
Gaseous carbon dioxide is the form in which the element carbon enters the plant and escapes from the animal. Within the organism it is dissolved in the tissue fluids, and hydrated to form carbonic acid, which in turn is neutralised by the buffer systems.

Under ordinary conditions of life, an adult man of 60–70 kg. excretes 750–900 gm. of CO_2 *per diem*. This corresponds to a level of 45–56 ml. CO_2 per 100 ml. arterial blood, and a CO_2 pressure in the alveolar air of the lungs of 40 mm. Hg.

Carbonic acid is the principal volatile acid produced in animal metabolism. It is sufficiently strong to form stable salts with the bio-metals, and yet sufficiently weak to constitute one of the buffer systems whereby the neutrality of the organism is maintained.

The reaction of the blood is being thrust continuously towards the acid side by the CO_2 liberated during metabolism, and this increase in acidity, in turn, serves to stimulate the respiratory mechanism whereby CO_2 is eliminated. Hence, this waste product is utilised as long as it is in the organism.

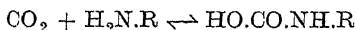
Carbon dioxide transport by the blood.—If pure water is exposed to carbon dioxide at a partial pressure of 40 mm. Hg, which is the usual alveolar value, the gas dissolves until the resulting solution of carbonic acid has a pH of 4.7 for ordinary temperature. If, however, blood be exposed to the same partial pressure of gas the resulting solution has a pH of about 7.4, which is only slightly less than the value for untreated blood. That is to say, blood has a considerable buffering power for H-ions. But blood is the transport medium for the elimination of carbon dioxide by the lungs, and must be provided with some mechanism for the rapid escape of the gas. When blood is exposed to reduced pressure carbon dioxide is released in two ways: (i) rapidly from the carbonic acid, and (ii) slowly from the acid carbonates in solution. The speed of the first reaction led Meldrum and Roughton to suspect the existence of a catalyst, which they later isolated from the red corpuscles, and named **carbonic anhydrase** because it catalysed the reversible reaction,



When blood is previously treated with dilute cyanide or other appropriate enzyme inhibitors, and then exposed to a vacuum, the amount of rapidly-released carbon dioxide is greatly but not entirely diminished, whereas the slowly-released carbon dioxide is unaffected.

From this it is concluded that carbon dioxide is transported in the blood in three distinct forms: (a) as stable acid carbonate, (b) as unstable carbonic acid, readily dehydrated by an enzyme, and (c) as an unstable salt, not attacked by the enzyme. This salt has been identified as a carbamate produced by union between CO_2 and an

uncharged $-\text{NH}_2$ group, such as occurs in hæmoglobin in slightly alkaline solution,



Carbamino
derivative

IONIC SYSTEMS

Ions are electrically charged particles formed by the spontaneous dissociation of acids, bases and salts, when dissolved in water or other ionising solvents. Ions may be classified according to sign as : (i) positively-charged *cations*, R^+ , which migrate to the cathode, or negative electrode ; (ii) negatively-charged *anions*, R^- , which migrate to the anode, or positive electrode ; and (iii) doubly charged dipolar, or *zwitter*-ions, $^+R^-$, which, when their charges are equal, migrate to neither electrode.

The principal biological ions are :—

- (1) *Metallic anions* : Na^+ , K^+ , Ca^{++} , Mg^{++} .
- (2) *Non-metallic anions* : H^+ , NH_4^+ , dipolar ions in acid solution.
- (3) *Non-metallic cations* : Cl^- , HCO_3^- , H_2PO_4^- , aliphatic and other acid radicles (R.COO^-), dipolar ions in alkaline solution.
- (4) *Dipolar ions* : Proteins and amino acids ($^+\text{H}_3\text{N.R.COO}^-$).

The other biological elements, such as Mn, Fe, Cu and I, occur either as compounds or in very low ionic concentration.

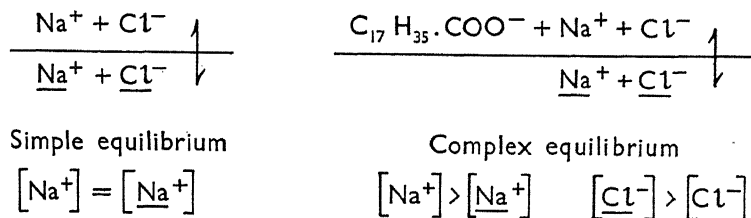
The ionic composition of the tissues and tissue fluids determines : (a) conductivity, (b) buffering power and pH, (c) oxidation-reduction conditions, (d) membrane potential and, in part, (e) osmotic pressure.

All *metazoa* may be regarded as organised aggregations of liquid cells maintained by a *milieu* of vascular and intracellular solutions. The bio-physical properties of the cell membrane presents many problems. According to Höber (1936), membrane phenomena may be referred to at least three types of structure, (i) sieve-like surfaces of differential permeability, which separate solutes according to particle size ; (ii) solvent-like surfaces, which dissolve appropriate solutes, and so permit entry to, or egress from the cell ; (iii) surface films of monomolecular dimension.

In a typical cell it is possible that all three types are represented, and constitute a **surface mosaic**, the pattern of which may vary with cell condition. Cell membranes, though relatively or absolutely impermeable to metallic ions, are permeable to water, food solutes and outgoing waste-products, and the intracellular pressure and cell volume are determined by the relative concentration of the solutes on either side of the membrane.

Chloride Shift.—Hamburger observed that when carbon dioxide enters the blood there is an accompanying migration of Cl^- from plasma to corpuscles. Conversely, when carbon dioxide escapes from blood there is a migration of Cl^- from corpuscles to plasma, although this change takes place against the concentration gradient for Cl^- . In outline, this "chloride shift" is due to (a) diffusion of carbonic acid into the corpuscle, where it is ionised by the intracellular buffer systems, which accept H^+ from the acid; (b) accumulation of HCO_3^- ions within the corpuscle until their concentration exceeds that of the HCO_3^- ions in the plasma; (c) diffusion of HCO_3^- ions from corpuscle to plasma, where they displace a corresponding number of Cl^- ions, which enter the corpuscle to balance the H^+ ions derived from the carbonic acid.

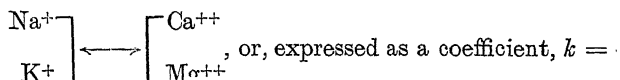
The Donnan Membrane Effect.—When a simple electrolyte, such as sodium chloride, is separated from its solvent by a permeable membrane, ionic diffusion takes place until equilibrium is reached, when the product of the concentrations of the ions on either side of the membrane is the same, or $[\text{Na}^+] \times [\text{Cl}^-] = [\text{Na}^+] \times [\text{Cl}^-]$. Furthermore, by analysing samples from each side, it can be shown that the sodium concentration $[\text{Na}^+]$ on one side equals that on the other side of the membrane, $[\text{Na}^+]$, and also that $[\text{Cl}^-]$ equals $[\text{Cl}^-]$. If, however, a non-diffusible ion, such as a stearic acid radicle, $\text{C}_{17}\text{H}_{35}\cdot\text{COO}^-$, be present on one side of the membrane it will inhibit the migration of some of the ions of the opposite sign, in this instance, Na^+ . When equilibrium has set in, $[\text{Na}^+] \times [\text{Cl}^-] = [\text{Na}^+] \times [\text{Cl}^-]$, as before; but now $[\text{Na}^+]$ is greater than $[\text{Na}^+]$ and consequently $[\text{Cl}^-]$ must be greater than $[\text{Cl}^-]$.



This unequal concentration of the ions leads to a difference in potential between the solutions on either side of the membrane, which can be calculated or estimated electrometrically. The phenomenon was first predicted and experimentally verified by F. G. Donnan, in 1911, and is generally known as the Donnan Effect. It has been applied to the study of protein systems by J. Loeb.

The physiological consequences of membrane potentials and ionic displacements are very great, and these processes modify or determine many forms of activity, including muscle contraction, nerve conduction, gland secretion, as well as general cell growth.

Once a cell has reached maturity it is, as a rule, impermeable to metallic ions, and depends on its ionic inheritance for the discharge of many of its functions. These, in turn, are regulated by the ionic pattern of the environment, the constituents of which enter into various synergic and antagonistic combinations whereby their physiological properties are enhanced or depressed. Thus, a balance operates between the four ions, Na^+ , K^+ , Ca^{++} and Mg^{++} , which may be written :—



Alteration in the concentration of any one ion, within limits, can be balanced by alteration in the concentration of one of the other ions, and as long as the coefficient k is unchanged the physiological properties of the solution will remain unaltered, at least as regards its effect on a particular tissue.

The recognition of metallo-ionic balance began with the work of Ringer on the maintenance of the heart beat by perfusion.

For the mammalian heart, a perfusion fluid must contain approximately : NaCl , 0.9 per cent. ; KCl , 0.04 per cent. ; CaCl_2 , 0.024 per cent. ; and NaHCO_3 , 0.01–0.03 per cent. This mixture constitutes Locke's solution.

Na^+ maintains the beat.

Ca^{++} affects the contraction process, and if excessive causes a decrease in relaxation, and ultimate cessation in a state of extreme contraction or "calcium rigor."

K^+ is antagonistic to Ca , and if excessive causes a weakening of the beat, and cessation in a state of complete relaxation. Cl^- , chiefly as NaCl , maintains the osmotic pressure of the fluid. NaHCO_3 forms a buffer system to keep the pH on the side of slight alkalinity. Rise in $[\text{H}^+]$ causes increased relaxation and weakening of beat ; fall in $[\text{H}^+]$ causes decrease in relaxation.

At present it is only possible to apply the principle of ionic balance in a general way when several different species of ions are concerned, but it has been worked out more fully for growth conditions in many land and water plants (Osterhout, 1906–1927).

The Inland Sea.—In 1889, Bunge, the Swiss chemist, outlined a theory to explain the occurrence and distribution of the biological metals. Life, he assumed, arose in the tepid waters of a primæval ocean, and during the slow evolution of the invertebrates and primal

vertebrates the tissues became adapted to a saline environment. When life eventually migrated from sea to land, the later vertebrates carried the chemical legacy of a marine ancestry. In the ocean the concentration of sodium chloride exceeds that of any other solute ; consequently, it is the chief inorganic constituent of the tissue fluids of the animal. The theory was developed by Quin ton (1897, 1912), who assumed, somewhat hastily, that all the inorganic constituents of blood-plasma and sea water were the same in nature and relative proportions, and that sea water when diluted until isotonic with the plasma of the animal is the correct physiological saline. That is to say, the internal medium of life is not water, but sea water—a concept anticipated with more magnificence by Algernon Swinburne :—

“ —With heart's thanksgiving
That in my veins like wine
Some sharp salt blood of thine,
Some springtide pulse of brine
Yet leaps up living.”

In 1904, Macallum independently reached the conclusion that “ the blood-plasma of vertebrates and invertebrates with a closed circulatory system is, in its inorganic salts, but a reproduction of the sea-water of the remote geological period in which the prototype representative of such animal forms first made their appearance.”

He supported his conclusion by a series of analyses of blood-serum and sea-water :—

Solution.	Percentage of Element.				
	Na	Ca	K	Mg	Cl
Sea water . . .	1·072	0·042	0·038	0·136	1·932
Human serum . . .	0·302	0·009	0·020	0·002	0·389
Sea water, diluted 1 : 3	0·357	0·014	0·012	0·045	0·644

The marked difference between the high concentration of magnesium in sea water and its low concentration in all vertebrate tissue fluids is explained by Macallum as being due to the selective action of the kidney, which stabilised the composition of the tissue fluids at a time when the magnesium content of the ocean was less than its present value.

Macallum's conception of a palæochemical factor at work in the organism to-day has been criticised by Dakin (1931) and by Pantin (1931).

Dakin observes that the composition of a tissue fluid represents the combined effects of a number of physiological processes, that in turn depend on the existence of membranes separating the cells

from the internal and the external environments: "We can only accept the universal saline composition of the blood of *metazoa*—with its variations—as a possible reflection of a long-continued existence and probable origin in a saline medium."

If all the body chloride exists in true solution as Cl^+ , it can be calculated that extracellular water makes up 26–28 per cent. of human body weight; intracellular water varies from 29 to 45 per cent., being lower in obese subjects.

Osmotic difference between the body fluids and the environment is maintained in order to preserve the inorganic pattern of the cells, and probably depends more on the requirements of cytoplasmic metabolism than on the conditions imposed by an immemorial marine ancestry.

GENERAL REFERENCES

- ATKINS, W. R. G. (1916). "Researches in Plant Physiology." London.
- BARCROFT, J. (1934), "Features in the Architecture of Physiological Function." Oxford.
- BJERRUM, N. (1935), "Acids, salts and bases." *Chem. Rev.*, **16**, 287.
- CLARK, W. M. (1928), "Determination of Hydrogen Ions." London.
- CLOSE, H. G. (1934), "Chloride and water in tissue constitution." *Biochem. J.*, **30**, 967.
- DONNAN, F. G. (1924), "Theory of membrane equilibria." *Chem. Rev.*, **1**, 73. "Symposium on indicators," (1935). *Chem. Rev.*, **16**, 53.
- DRINKER, C. K. and M. FIELD (1933), "Lymphatics, Lymph and Tissue Fluid." London.
- GORTNER, R. A. (1937), "Outlines of Biochemistry." Ed. II. New York.
- HARVEY, H. W. (1928), "Biological Chemistry and Physics of Sea Water." London.
- LOEB, J. (1906), "Dynamics of Living Matter." New York.
- LOEB, J. (1916), "The Organism as a Whole." New York.
- LYALL, A. (1939), "Pathology of Chloride Metabolism in Man." *British Med. J.*, **ii**, 760.
- MACALLUM, A. B. (1926), "Palæochemistry of the body fluids and tissues." *Physiol. Rev.*, **6**, 316.
- MCCANCE, R. A. (1936), "Experimental sodium chloride deficiency." *Proc. Roy. Soc., B*, **814**, 245.
- MICHAELIS, L. (1930), "Oxidation-reduction Potentials." New York.
- PANTIN, C. F. (1931), "Origin of the composition of the body fluids in animals." *Biol. Rev.*, **6**, 459.
- ROBERTSON, J. D. and WEBB, D. A. (1939), "Micro-analysis of sea water and body fluids." *J. Exper. Biol.*, **16**, 155.
- VOGEL, A. I. (1939), "Quantitative Inorganic Analysis," London.

CHAPTER IV

SOLUTIONS AND COLLOIDAL SYSTEMS

ALL the chemical reactions associated with life take place between substances in aqueous solution or associated with aqueous colloidal systems, and the properties of these solutions and systems largely determines tissue organisation and function.

Solutions are micro-homogeneous mixtures the composition of which may vary within certain limits of saturation.

The characteristic of a micro-homogeneous mixture is that its constituents cannot be separated by mechanical methods, such as sedimentation or filtration. In the nomenclature of physical chemistry, a true solution is *monophasic*, all its constituents being present as part of the same physical state, or *phase*.

The constituent present in excess is termed the *solvent*, the other constituents being the *solutes*. In a true solution the solvent is usually a liquid, but may be a solid, as in the so-called "solid solutions" represented by glasses and alloys. The solutes, or dissolved constituents, may be gases, liquids or solids, the ultimate state of the solution (liquid or solid) being determined by the state of the solvent. The composition of a solution is limited by the solubility of the solutes, a critical point being reached when the solution is saturated in regard to a particular solute, the excess of which remains undissolved or separates out from the saturated solution, and may be removed by sedimentation or filtration. These heterogeneous mixtures are said to be *polyphasic* in that they contain mechanically-separable forms of matter.

Structure.—The solute particles in a solution may be present as non-ionised molecules, such as sugar, or as electrically charged ions, three types of which exist : (i) positively charged cations, such as H^+ , Na^+ , and Ca^{++} ; (ii) negatively charged anions, such as OH^- and Cl^- ; and (iii) dipolar, or zwitter-ions, such as amino acids, $+R^-$, which carry charges of opposite electric sign.

Conductivity.—The electrical conductivity of the solution is determined by the nature and concentration of the solute particles. Solutions of non-ionised molecules or dipolar ions have minimal conductivity and are termed non-electrolytes ; solutions containing ions have a conductivity depending on the ionic concentration, and are termed electrolytes.

All aqueous solutions have a low residual conductivity irrespective of the nature of the added solutes, owing to the fact that water itself is slightly ionised into H^+ and OH^- , and thus generates ionic solutes.

Properties of Solutions.—In addition to the properties due to the solvent and the solutes, solutions display characteristics which may be described as emergent, since they are not shown by the individual constituents; of these, the most familiar is osmotic pressure.

Osmotic Pressure.—Solution is a spontaneous process and tends to occur whenever a solute is added to an appropriate solvent. If, however, a solute or a concentrated solution be separated from a solvent by means of a *semipermeable membrane* the tendency of the solute particles to dissolve is shown by the pressure they exert on the membrane. This force, or *osmotic pressure*, can be measured in various ways, and affords information as to the number of solute particles present in a given volume of solution.

Semipermeability implies that a surface allows the passage of the solvent but restricts the passage of the solute responsible for the osmotic pressure. Semipermeability varies greatly with material structure. A filter paper is permeable to all solutes present in true aqueous solution, and also permits the passage of colloidal mixtures, such as milk and egg albumin. An animal membrane, such as parchment or peritonæum, will restrict the passage of proteins, soaps and other solutes of large molecular dimensions. Thin membranes can be constructed of collodion, cellophane, or gelatine of such degree of selectivity as to be permeable to ions but not to simple molecules.

Estimation of Osmotic Pressure.—(i) *Direct.*—The solution of known concentration is enclosed in a semipermeable container attached to a manometer tube. The container is immersed in a vessel containing an excess of the solvent, some of which diffuses into the container causing a rise of the solution in the manometer tube. When equilibrium is reached, the osmotic pressure of the diluted solution inside the container is balanced by the hydrostatic pressure of the liquid in the manometer, and can be measured in atmospheric units. Modifications of the apparatus include the use of a mercury manometer to avoid excessive dilution of the mixture, and the employment of metallic containers with frames to support the membranes.

(ii) *Indirect.*—The boiling point of a solvent is raised and the freezing point is depressed by addition of solutes, the extent of the change being proportional to the concentration of solute particles.

It can be shown from the gas laws that an aqueous solution of a non-ionising solute in molecular concentration (1 gm. molecular equivalent of solute in 1 litre of solvent) has an osmotic pressure of

22.4 atmospheres at 0° , a boiling point of 100.52° , and a freezing point of -1.86° . Consequently, if the molecular weight of a non-ionising solute, such as a sugar, is known, it is possible to calculate the osmotic pressure, boiling point and freezing point of a solution of any given concentration.

Similarly, knowing the concentration of the solute it is possible to calculate the molecular weight from any one of the three properties of the solution. In practice, the easiest estimation is that of the freezing point, which is determined in a *cryoscope*, in which the value of Δ , the depression of the freezing point, is read by means of a sensitive Beckmann thermometer.

$$\text{Osmotic pressure, in atmospheres} = \frac{22.4 \times \Delta, \text{ in } ^{\circ}\text{C}}{1.86}.$$

The molecular weight, M , of a single solute can be calculated from the depression of the freezing point, Δ , of a solution containing w gm. of solute per litre, since

$$M = \frac{1.86 \times w}{\Delta}.$$

Solute.	M .	Osmotic Pressure of a 10 per cent. Solution.	Freezing Point of a 10 per cent. Solution.
Urea . . .	60	37.34 atm.	-3.1°
Glucose . . .	180	12.4 ..	-1.03°
Fructose' . . .	180	12.4 ..	-1.03°
Sucrose . . .	342	6.5 ..	-0.54°

The osmotic pressure of the blood plasma and tissue fluids is determined by a variety of solutes, the chief of which are Na^{+} and Cl^{-} , and is maintained in the region of 7 atmospheres (corresponding to a freezing point of -0.56°) by the intake of water and the excretion of urine, the osmotic pressure of which may vary between 12 and 24 atmospheres during the day.

COLLOIDAL SYSTEMS

Intervening between true solutions and unstable suspensions, which can be separated by simple mechanical methods, is a class of solutions termed *colloidal* by Graham, in 1856, to denote their glue-like non-crystallisable nature, as distinct from that of the *crystalloids*, or solutes present in true solutions. By selective *dialysis*, or filtration through parchment, he was able to separate colloids from accompanying diffusible crystalloids. Subsequently, it was found that the intrinsic difference between the two groups

depended on the size of the solute particles and not on the crystallisability, and Ostwald suggested that colloids represented a state of matter rather than a class of compounds. In a true solution, the solute particles are less than $1\text{ m}\mu$ in diameter; in a colloidal solution the particles range from $1\text{ m}\mu$ to $100\text{ m}\mu$ in diameter; in suspensions and emulsions the particles are greater than $100\text{ m}\mu$ in diameter.¹

Colloidal solutions resemble true solutions in that they are homogeneous and are not resolved by simple filtration or sedimentation. The dispersed particles, however, display surface properties not shown by true solutes, and also may be separated by special methods involving the high-speed centrifuge or by ultra-filtration, and for this reason colloids are sometimes termed polyphasic solutions, the term phase being used to denote a mechanically separable form of matter.

To emphasise the distinction, the particles in a colloidal system are termed the *disperse* or *internal phase*, the solvent being the *continuous* or *external phase*.

Classification of Colloidal Systems.—Unlike true solutes, the various components of a colloidal system can be any one or more of the three states of matter.

Representing the system as *solute/solvent*, the following arrangements are possible: gas/liquid, gas/solid, liquid/gas, liquid/liquid, liquid/solid, solid/gas, solid/liquid, solid/solid.

(i) *Gas/liquid* systems are represented by foams and froths, the permanence of which depends on the presence of stabilisers, such as soaps and saponin glucosides, which form froths when their aqueous solutions are shaken up with air.

Froth production is rare in nature, apart from fermentations, but an interesting example is afforded by the plant parasite which protects itself by means of a foam derived from leaf sap. Pathological and often fatal examples of froth formation in the higher animal are seen in air embolus, and pulmonary oedema.

(ii) *Gas/solid* systems in which gaseous particles are distributed through a solid external phase are of industrial importance in connection with the absorption of vapours by means of charcoal filters, and their study has been encouraged by the introduction of gas as a weapon in civilised warfare. Many porous substances, including spongy platinum and palladium, adsorb and subsequently dissolve gases.

(iii) *Liquid/gas* systems, represented by mists and fogs, occur temporarily when air saturated with moisture is cooled suddenly. The stability of a mist depends on the size of the particles, being

¹ $1\text{ mm.} = 1,000\text{ }\mu$ (microns). $1\text{ }\mu = 1,000\text{ m}\mu$ (millimicrons).

greatest when they are small, but stabilising factors, such as products of coal combustion, also operate to preserve the characteristic atmosphere of industrial towns in damp weather.

Cigarette smoke provides an example of two colloidal systems. The bluish smoke from the combustion is a dispersion of carbon particles carried upwards by the current of heated gas. The brownish exhalation is a fog formed by salivary moisture stabilised by products of tobacco combustion.

(iv) *Liquid/liquid* systems are termed *emulsoids* to distinguish them from unstable emulsions. They are an important class of biological colloids, and represent the form in which liquid fats are transported or secreted by the organism. Milk is a 3-4 per cent. solution of fat, which is in an emulsoid form stabilised by milk protein.

(v) *Liquid/solid* systems, or *gels*, are represented by natural and artificial jellies and mucoids, and by butter, all of which consist of a solid external phase retaining a dispersed liquid phase in its interstices.

(vi) *Solid/gas* systems are represented by colloidal smoke, the stability of which depends on the fineness of the particles and the presence of stabilisers.

(vii) *Solid/liquid* systems, sols or *suspensoids*, constitute the principal biological colloids, and are represented by the soluble proteins of the tissues and tissue fluids, and similar compounds of high molecular weight.

Metallic sols of gold, silver, copper, etc., form an important class of suspensoids, and are used industrially and therapeutically in many ways.

(viii) *Solid/solid* systems are often included among true solid solutions, but are of little biological interest, although it is possible to attribute the structure of bone and other metallo-skeletal tissue to the formation of solid solutions from calcium salts.

Properties of Colloidal Systems.—Colloidal solutions resemble true solutions in that they exert an osmotic pressure and have a lower freezing point than that of the solvent. Neither effect is as marked as in the true solution owing to the relatively greater size and smaller concentration of the dispersed particles. The osmotic pressure of the plasma proteins, however, is an important factor in maintaining the volume of blood, and in man has a normal value of 305-307 mm. H₂O (0.03 atmospheres) at 22° C. In addition to these general properties, colloidal systems, especially suspensoids, exhibit special properties due to the surface and surface charge of the dispersed particles. These properties are:—

(1) *Faraday-Tyndall Effect*.—When a convergent beam of light

is sent transversely across a true solution, and examined by an observer at right-angles to the path of the beam, the solution appears optically empty. When the observation is repeated using a colloidal solution, light is reflected from the surface of the disperse particles, and the path of the beam is seen as an illuminated cone. By using a strong source of light, colloidal particles can thus be detected in very low concentration, and the method is applied industrially for this purpose.

A similar method of transverse illumination is used in the ultra-microscope whereby particles smaller than the shortest wave-length of visible light ($400\text{ m}\mu$) are revealed as radiating points against a dark background.

When strongly illuminated by light of short wave-length, it has been found that molecules in true solution are able to scatter sufficient of the radiation to enable their structure to be studied spectroscopically. This *Raman effect* is employed in the elucidation of molecular architecture.

(2) *Brownian Movement*.—When examined microscopically against a dark background, colloidal particles are observed to be in continual rapid irregular motion, a phenomenon first recorded in 1827 by the botanist R. Brown. Brownian movement is a form of perpetual motion, and is due to bombardment of the disperse particles by the molecules of the solvent.

(3) *Migration in an Electric Field, Electrophoresis*.—Dispersed particles carry an electric charge, and migrate to the anode or the cathode when the solution is electrolysed. Typical positively charged colloids (cathions) are : proteins in acid solution, hæmoglobin, ferric hydroxide and aluminium hydroxide suspensoids. Typical negatively charged colloids (anions) are : proteins in alkaline solution, starch, soaps and metallic sols.

The colloidal charge is altered by addition of electrolytes, especially acids or bases, and may be neutralised or reversed owing to combination between the colloid and solute ions. A neutral disperse phase is said to be iso-electric, in which condition it migrates neither to anode nor to cathode.

(4) *Adsorption at the Phase Interface*.—Solute that lower surface tension tend to accumulate on the free surfaces of colloidal particles. This surface condensation is termed adsorption, and is of special significance in colloidal systems because of the enormous area presented by the disperse phase. Colloids often display preferential absorption for a particular class of solutes, and one solute may compete with another solute and displace it from the adsorbing surface.

This is exemplified in the use of tartrazol as an adsorption indicator in the estimation of chlorides (p. 394).

As a result of adsorption, the solute, or *adsorbate*, becomes highly concentrated on the colloid surface, and may undergo subsequent changes due to chemical reaction with the colloid. Adsorption is a preliminary stage in enzyme catalysis, and is of primary importance in regulating the distribution of solutes among the tissue surfaces that make up the framework of structures associated with life.

Colloidal Stability.—The stability of a colloidal system depends on the combined effect of four factors :—

(1) *Diameter of Disperse Particles.*—The sedimentation rate of a particle falling in a liquid may be found by means of Stokes's law :

$$V = \frac{2}{9} \frac{r^2 (s - s')g}{u},$$

where V = rate of fall,
 r = radius of particle,
 s = density of particle,
 s' = density of continuous phase,
 g = gravity constant (981),
 u = viscosity of solution.

From this it will be seen that rate of sedimentation is proportional to the square of the radius, and if the particle be sufficiently small, months, years, or even centuries, will be required for complete sedimentation.

(2) *Brownian movement* tends to keep the particles distributed throughout the system.

(3) *Electric surface charge* tends to keep the particles from flocculating and precipitating, since bodies of like charge are mutually repellant. The importance of the surface charge is shown by the fact that colloids are least stable when at the isoelectric point.

(4) *Surface stabilisation.* Metallic sols, although they have a disperse phase of very small dimensions, are so unstable that they are classified as *lyophobic*, owing to the readiness with which they lose their solvent phase. Emulsoids and organic suspensoids, on the contrary, are examples of *lyophil* or hydrophil colloids, and are usually very stable. The difference is attributed to adsorption of the solute by the lyophil particles.

Emulsifiers and Stabilisers.—These are substances which when added in small quantities promote the formation of colloidal systems and render them less liable to spontaneous precipitation. Gum acacia, gum tragacanth, soaps, saponins, gelatin and lecithin are representative stabilisers used industrially. Egg yolk, on account of the lecithin it contains, is used in pharmaceutical and in domestic preparations, such as the manufacture of mayonnaise by emulsification of vinegar in oil.

Precipitation and Coagulation of Colloids.—Any operation that removes the stability factors in a colloidal system tends to bring

about aggregation of the disperse particles. Violent agitation, as in the churning of milk, addition of colloids or electrolytes of the opposite electric charge, freezing and thawing, all promote precipitation of colloidal particles. An important example is the irreversible coagulation that takes place when certain higher proteins are heated in slightly acid solution (p. 151).

Analysis of Colloidal Systems.—The average dimension of the disperse phase particles may be found in several ways, the most important of which are :—

(1) *Ultra-filtration* through surfaces of standard porosity. The filters usually employed are collodion, cellophane and gelatin. From the strength of a gelatin filter the size of the pores may be calculated, and depend on the concentration of gelatin present, irrespective of the thickness of the filter.

(2) *Rate of sedimentation* may be measured by means of a sedimentation balance, one pan of which is immersed in the solution, and acquires weight from the subsidence of the particles. The radius of the particles is then calculated by means of Stokes's law. The method is only applicable to suspensions and coarser suspensoids that subside rapidly.

Average Diameter of Dispersed Particles

Type.	Visibility.	Example.	Diameter.
Molecular	Not visible by ultra-microscope	True solutes	0.5 μ —5 μ
Ultra-microscopic	Not visible by microscope	Colloidal solutes	5 μ —100 μ
Microscopic	Not visible by the unaided eye	Fine suspensions	200 μ —1 μ
Directly visible		Coarse suspensions	> 10 μ (0.01 mm.)

0.1 μ .	1 μ .	10 μ . 100 μ .	1 μ .	10 μ .	100 μ . 1 mm. (1,000 μ).
True solutions			Colloids		Suspensions
Particles pass through ordinary filter paper			Precipitates		
Brownian movement			Particles retained by ordinary filter paper		
			No Brownian movement		

(3) *Ultra-centrifugal Methods.* By means of a high speed centrifuge rotating at 10,000 to 400,000, or more, revolutions per minute, it is possible to submit colloidal systems to forces sufficient to separate the dispersed phase. The sedimentation rate is found by observation of the meniscus of separation, and from this the radius of the particles is calculated. The method has been used successfully by Svedberg for the estimation of the diameter and weight of protein molecules (p. 144).

Methods of Preparing Colloidal Solutions.—(1) *Use of a suitable solvent.*—Many organic compounds, such as proteins, soaps, and gums, form colloidal solutions spontaneously when treated with water, and occur as colloidal systems in their natural conditions.

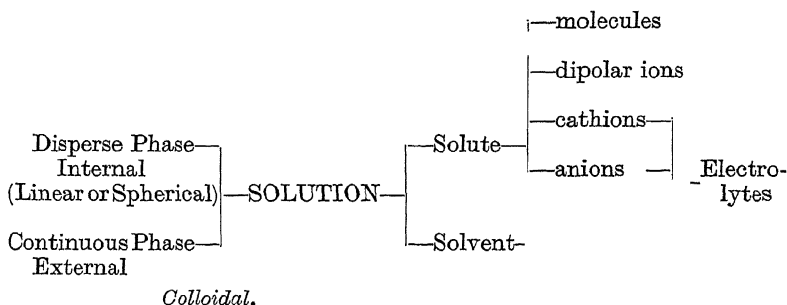
(2) *Condensation of True Solute Particles.*—By reduction or hydration it is possible to aggregate the ions of various metals so that they form particles of colloidal dimension and pass from the true to the colloidal state.

(3) *Dispersion, or Peptisation, of Insoluble Particles.*—By mechanical disintegration in a colloid mill it is possible to render insoluble substances, such as graphite or chalk, sufficiently fine to form colloidal solutions in water, oil and other liquids. While it is also possible by means of an electric arc formed between electrodes of a metal, immersed in water or other liquids, to disperse the metal in the form of a sol. These are industrial methods used for preparing colloidal lubricants and therapeutic agents.

Colloidal particles can be classified according to shape as linear and spherical. Linear colloids, such as cellulose, are fibrous, and dissolve with much swelling to form viscid solutions. Spherical colloids, such as glycogen, are powders in the solid state, and give solutions of low viscosity.

Summary

True Solutions.	Colloidal Solutions.
<ol style="list-style-type: none"> 1. One phase (mechanically homogeneous). 2. Optically empty. No Faraday-Tyndall cone. 3. Solute particles less than 1-5 μ in diameter. 4. No Brownian movement. 5. Solute particles show no surface properties other than the Raman effect. 	<p>More than one phase (mechanically heterogeneous). Optically dense. Faraday-Tyndall cone when illuminated transversely. Disperse particles range from 5 μ to 100 μ. Particles show Brownian movement. Disperse particles show surface properties and carry a surface charge.</p>



GENERAL REFERENCES

- ALEXANDER, J. (1937), "Colloid Chemistry." 4th Ed. London.
 BEUTNER, R. (1933), "Physical Chemistry of Living Tissues." London.
 BUZAGH, A. (1937), "Colloid Systems." (*Trans. Darbishire.*) London.
 FERRY, J. D. (1936), "Ultrafilter membranes and ultrafiltration."
Chem. Rev., **18**, 373.
 FREUNDLICH, H. (1926), "Colloid and Capillary Chemistry." London.
 HOLMES, H. N. (1932), "Laboratory Manual of Colloid Chemistry."
 New York.
 LANGMUIR, I. (1933), "Surface chemistry." *Chem. Rev.*, **13**, 147.
 LLOYD, D. JORDAN (1932), "Colloid structure and its biological
 significance." *Biol. Rev.*, **7**, 254.
 MICHAELIS, L., and P. RONA (1933), "Practical Physical and Colloidal
 Chemistry for Students of Medicine and Biology." (*Trans. T. R.
 Parsons.*) Cambridge.
 SVEDBERG, T. (1934), "Sedimentation of molecules in centrifugal
 fields." *Chem. Rev.*, **14**, 1.

PART II

ORGANIC BIOCHEMISTRY

"Bear with me, gentlemen, while I remind you
of the incessant molecules that bind you."

HUMBERT WOLFE.

CHAPTER V

CLASSIFICATION AND CHARACTERISTICS OF ORGANIC COMPOUNDS

WHILE the greater part of the tissues and tissue fluids of the organism is inorganic in that it is composed of water and dissolved salts, the colloidal matrix of the cells, secretions and vascular fluids is organic in that it is made up of compounds containing carbon and hydrogen as structural elements. In addition, the manifold chemical transformations and exchanges that reveal the operations of life are concerned chiefly with the decomposition, elaboration, storage and excretion of organic compounds.

Living organisms may be divided into two classes : the *autotrophes*, represented by green plants and some highly specialised bacteria ; and the *heterotrophes*, represented by animals and by plants free from chlorophyll. Autotrophic organisms are able in the presence of sunlight to synthesise their organic constituents from an inorganic environment providing water, carbon dioxide, nitrate, ammonia and the additional biological elements. Heterotrophic organisms require organic food materials as well as water, oxygen and the biological elements. In the collaboration that has made animal life possible on earth, the autotrophes act as collectors and storers of energy in chemical form available for the nutrition of the heterotrophes.

The bio-organic compounds associated with plants and animals are wide in variety and often great in complexity, and no classification yet proposed is entirely logical. Features of chemical structure, physiological significance and biological distribution are selected according to convenience.

Classification by Structure.

1. Carbohydrates.
2. Proteins.
3. Lipides.
4. Steroids.
5. Porphyrins.
6. Purines.
7. Pyrimidines.
8. Carotinoids.
9. Terpenes.
10. Flavins.
11. Flavones.
12. Alkaloids, etc.

Classification by Function.

1. Plastics.
2. Nutrients.
3. Hormones.
4. Catalysts.
5. Vitamins.
6. Pigments.
7. Buffers.
8. Storage products, etc.

Neither of these classifications is exclusive or exhaustive, and each merely serves to emphasise the biological significance of the included compounds.

ORGANIC TYPE FORMULÆ

Organic compounds, other than symmetrical hydrocarbons, can be represented as being composed of a linear or cyclic carbon nucleus or *radicle*, R, carrying one or more reactive groups or side-chains. The radicle confers stability and physical characteristics on the compound; the side-chains determine chemical reactivity. Bio-organic radicles are represented chiefly by phenyl, C_6H_5- , and indolyl, C_8H_6N- , rings, neither of which can be assembled by the higher animal, but must be provided in the diet. Reactive groups are represented chiefly by hydroxyl, $-OH$, carboxyl, $-COOH$, aldehyde, $-CHO$, keto, $=CO$, and amino, $-NH_2$, configurations.

(1) Systems Present in Biological Compounds.

(a) Derivatives of Open-Chain, or Linear Hydrocarbons.

Parent Hydrocarbon.

 Methane, CH_4

 Ethane, C_2H_6

 Propane, C_3H_8

 Butane, C_4H_{10}

 Pentane, C_5H_{12}

 Hexane, C_6H_{14}

Radicle.

 Methyl, *Me*, CH_3- , Methylene, $CH_2=$

 Ethyl, *Et*, $CH_3.CH_2-$, Vinyl, $CH_2.CH=$

 Propyl, *Pr*, $CH_3.(CH_2)_2-$

 Allyl, $CH_2:CH.CH_2-$

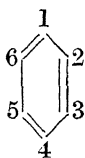
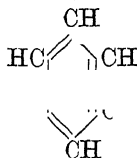
 Butyl, $CH_3.(CH_2)_3-$

 Amyl, $CH_3.(CH_2)_4-$

 Hexyl, $CH_3(CH_2)_5-$

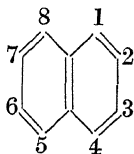
(b) Derivatives of Closed-Chain, or Iso-Cyclic Hydrocarbons.

Parent Hydrocarbon

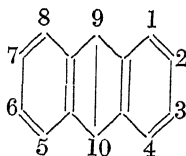
 Benzene, C_6H_6


Benzene.

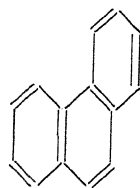

 ortho (1 : 2), meta (1 : 3) para (1 : 4)
Disubstitution Derivatives.



Naphthalene.

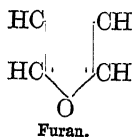
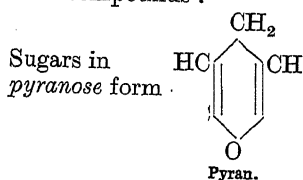


Anthracene.



Phenanthrene.

(c) *Heterocyclic Compounds*.—Cyclic structures containing other elements in the ring in addition to carbon and hydrogen are termed heterocyclic, and are represented biologically by the following parent compounds :—

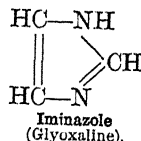
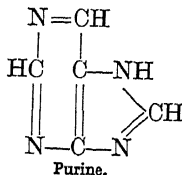
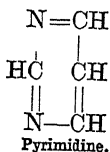
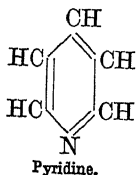


Sugars in
furanose form

Prophyrins
Blood pigments
Bile pigments
Chlorophyll



Pyrrole.

Indole
(Benzpyrrole).

(2) Groups and Linkages Present in Biological Compounds.

Primary alcohol, $-\text{CH}_2.\text{OH}$

Secondary alcohol, >CH.OH

Amino, $-\text{NH}_2$ attached to
 $-\text{CH}_2-$

Amido, $-\text{NH}_2$ attached to
 $-\text{CO}-$

Carbamido, or uramido,
 $-\text{NH.CO.NH}_2$

Guanidino, $-\text{NH.C(NH).NH}_2$

Aldehyde, $-\text{CHO}$
Carboxyl, $-\text{COOH}$

Ketone, >CO

Imino, >N-

Ester linkage, $-\text{CH}_2.\text{CO.O.R}$

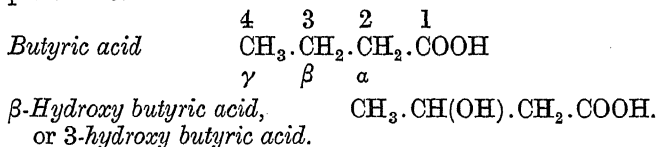
Peptide linkage,

Disulphide linkage,

$-\text{CH-S-S-CH}_2-$

Thiol, or sulphhydryl group, $-\text{SH}$

The position of a substituent group in a linear compound is denoted by numbering the C atoms from right to left, or by the use of a Greek alphabet, the C atom next the characteristic group in the compound being described as the α -carbon. Confusion is liable to arise between the two types of nomenclature, and the numerical form is preferable.



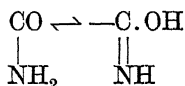
The position of a group in a cyclic compound is denoted numerically in reference to a particular carbon atom, C¹.

Double bonds, —C=C— , in organic compounds are denoted by the symbol Δ with a pair of suffixes to indicate the position number of each C atom linked by the double bond.

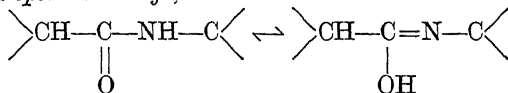
The chief reactions undergone by organic compounds include *oxidations*, *reductions*, *condensations*, or union by elimination of water, *hydrolyses*, or resolution by addition of water, *polymerisation*, or combination of two or more similar molecules, *internal rearrangement* and *ring formation*.

Tautomerism, or dual configuration, is shown by certain organic groups and compounds, the structure of which differ under different conditions. Important biochemical examples are:—

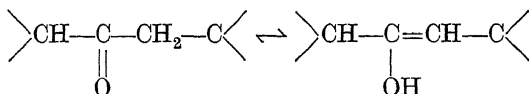
(a) *The Amide Group*,



The Peptide Linkage,



The Keto Compounds,



Assumption of the >C—OH , or *Enol* configuration is promoted by alkalis.

Assumption of the >CO , or *Keto* configuration is promoted by acids.

CHAPTER VI

THE CARBOHYDRATES, OR GLUCIDES

Definition.—Carbohydrates are neutral compounds composed of carbon, hydrogen and oxygen in which the H : O ratio is 2 : 1, as shown by the carbohydrate type formula $C_m(H_2O)_n$. Chemically, they are polyhydroxy alcohols with potentially active aldehyde or ketone groups. Analytically, they are all reducing sugars or give rise to reducing sugars on hydrolysis. Biologically, they represent one of the three principal forms in which carbon is transported, stored and utilised in living organisms.

Nomenclature.—Carbohydrates were originally classified as :

<i>Saccharides.</i>	<i>Polysaccharides.</i>
(The sugar group.)	(The starch-cellulose group.)
All soluble in water to form true solutions.	Higher members insoluble ; lower members form colloidal solutions in water.
Sweet taste.	No sweet taste.
Crystallisable.	Not crystallisable.
No colour reaction with iodine.	Higher members give blue or brown colour with iodine.
	All are resolved by hydrolysis into saccharides.

The name "carbohydrate" records the fact that in these compounds the H : O ratio is similar to that in water, H_2O . The name "glucide" indicates that all the members of the family are either sugars (or "glucoses") or compounds giving rise to one or more sugars on hydrolysis ("glucosides"). In modern nomenclature the name of each carbohydrate ends in the suffix "ose"; the prefix indicating the number of C atoms in the molecule. Thus, *hexose* denotes a glucide, or carbohydrate, containing six C atoms.

All simple sugars contain a potentially active *aldehyde*, $-CHO$, or *ketone*, $-CO-$, group; and in consequence may be termed *aldo-* or *keto-*sugars according to the nature of the group.

For classification, carbohydrates are now divided into :—

- (A) *Simple sugars*, or *monosaccharides*.
- (B) *Compound sugars*, composed of two or more monosaccharides.
- (C) *Polysaccharides*, or *polyhexosides*, formed by the polymerisation of several units of a monosaccharide.
- (D) *Heterosaccharides*, polysaccharides containing non-sugar residues.

(E) *Glycosides*, compounds of a sugar and a non-sugar-residue.

(F) *Saccharide derivatives*, oxidation, reduction and condensation products obtained from and reconvertible into sugars.

Classification of the Carbohydrates.—A. **Monosaccharides.**—Simple sugars having the general formula $C_nH_{2n}O_n$. All are soluble in water, have a sweet taste, and reduce alkaline solutions of copper.

- (1) Monose, CH_2O , *Formaldehyde*. Theoretically the first member of the series, but not regarded as a sugar, owing to absence of the appropriate physiological characters. It is believed to be a possible intermediate compound in the synthesis of carbohydrate in the plant.
- (2) Diose, $C_2H_4O_2$, *Glycolaldehyde*. The simplest sugar.
- (3) Trioses, $C_3H_6O_3$. *Glycerose*, an aldotriose, the reference sugar in classification. *Dihydroxyacetone*, a ketotriose.
- (4) Tetrose, $C_4H_8O_4$. Four aldotetroses and a ketotetrose are known.
- (5) Pentose, $C_5H_{10}O_5$. Eight aldopentoses are known, including *arabinose*, *xylose*, and *ribose*. In addition, six methyl-pentoses, or methylloses, have been found; their formula being $C_5H_9(CH_3)O_5$. *Rhamnose* is the most familiar.
- (6) Hexose, $C_6H_{12}O_6$. Sixteen aldohexoses and eight ketohexoses are known. The chief aldohexoses are *D-mannose*, *D-glucose*, and *D-galactose*; the chief ketohexose is *D-fructose*.
- (7) Heptose, $C_7H_{14}O_7$. Five heptose sugars are known.
- (8) Octose, $C_8H_{16}O_8$. Three octose sugars are known.
- (9) Nonose, $C_9H_{18}O_9$. Two members are known.
- (10) Decose, $C_{10}H_{20}O_{10}$. One member is known.

B. Compound Saccharides.—Sugars formed by union of two or more monosaccharides, and convertible into monosaccharides by hydrolysis.

- (1) *Disaccharides*.—The chief disaccharides belong to the class of dihexosides, or sugars formed by union of two hexose molecules, with elimination of one water molecule. The general formula is $(C_6H_{11}O_5)-O-(C_6H_{11}O_5)$, or, briefly, $C_{12}H_{22}O_{11}$. Ten dihexosides are known, the most important being :—

Reducing Hexosides.

Non-reducing Hexosides.

Maltose = glucose-glucoside

Sucrose = fructose-glucoside

Cellose = glucose-glucoside

Trehalose = glucose-glucoside.

Lactose = glucose-galactoside.

In the non-reducing sugars the aldehyde group participates in the linkage, and is not available for reducing the copper reagent.

- (2) *Trisaccharides*.—Sugars formed by condensation of three monosaccharides, with elimination of two water molecules. The general formula is $C_{18}H_{32}O_{16}$, examples being *raffinose* (galactose-~~galactose~~ glucose), and *melezitose* (glucose-glucose-fructose), both non-reducing sugars.
- (3) *Tetrasaccharides*, $C_{24}H_{42}O_{21}$.—*Stachyose* is the only example known.

C. Polysaccharides.—Carbohydrates of high molecular weight and colloidal dimensions. Like compound saccharides, they are formed from monosaccharide units, but, unlike compound saccharides, they have no typical sugar characteristics, such as sweetness and true solubility. Hence, it is assumed that in polysaccharides the sugar units are held together by some form of polymerisation, and not by simple condensation.

Polysaccharides are classified according to the monosaccharides they liberate on hydrolysis.

- (1) Pentosans, $(C_5H_8O_4)_n$. Polymers of pentoses. Examples are *araban*, from gum arabic, and *xylan*, from straw.
- (2) Hexosans, $(C_6H_{10}O_5)_n$. Polymers of hexoses. Important examples are: dextrin, starch, cellulose, glycogen, and lichenin, all of which are *glucosans*, or polymers of glucose. Inulin, a *fructosan*, or polymer of fructose. Mannan, a polymer of mannose.

D. Heterosaccharides.—Complex polysaccharides containing non-sugar residues in addition to saccharide units.

- (1) Heteropentosans. Gums, mucilages, pectic substances, hemicelluloses.
- (2) Heterohexosans. Lignocellulose, pectocellulose, lipocellulose, chitin.

E. Glycosides.—Compounds containing a saccharide and a non-sugar, or *aglucone*, residue. Many highly active drugs, such as digitalis and strophanthin, are glycosides.

When the saccharide residue is glucose the compound is often called a "glucoside," although in modern sugar chemistry this term is also applied to any compound saccharide containing glucose as one of its units.

F. Saccharide Derivatives.

- (1) Saccharide alcohols, or mannitols, formed by reduction of sugars.
- (2) Saccharide acids, formed by partial oxidation of sugars.
- (3) Saccharide esters.
- (4) Aminosaccharides, hexosamines.

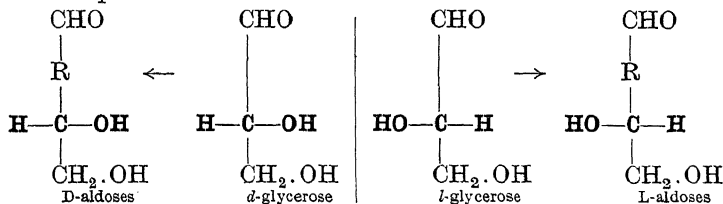
The Optical Properties of Carbohydrates.—All carbohydrates contain one or more asymmetric carbon atoms in which the carbon is united to four different kinds of components. This type of configuration endows the compound with (i) optical activity and (ii) optical or stereo-isomerism. By optical activity is meant the power possessed by the compound, both in crystalline form and in solution, of rotating in one or other direction a beam of plane-polarised light sent through it.

Optical activity is not directly observable in ordinary light, but requires the use of a polariscope which cuts out all the non-polarised light from the projected beam. To determine the direction and degree of rotation imparted to the beam, an analyser is fixed on the eye-piece of the polariscope, and can be turned in either direction until it compensates exactly for the rotation imparted to the light by the asymmetric compound. By this means the optical activity, expressed in terms of dextro-rotation (+) or lævo-rotation (—), to the clockwise right or anti-clockwise left of the observer, can be measured.

For every optically active dextro- or lævo-rotatory compound, a corresponding stereo-isomer, or epimer, exists, having the same formula but exactly opposite optical properties. This can only be explained by assuming that the groups attached to each asymmetric carbon atom can be arranged in either of two ways, and that the fact that chemical compounds occupy tri-dimensional space must be recognised in constructing formulæ.

When this is done, the structural formula of an optically active compound appears as the mirror-image of the formula of the corresponding epimeric compound.

All simple sugars can be regarded as derivatives of a primitive sugar, *glycerose*, $\text{CH}_2\text{OH}.\text{CHOH}.\text{CHO}$, got by partial oxidation of glycerol, $\text{CH}_2\text{OH}.\text{CHOH}.\text{CH}_2\text{OH}$. Glycerose contains an asymmetric C atom, and exists in two isomeric forms: *dextro*-glycerose and *lævo*-glycerose. Sugars lineally derived from the *dextro*-glycerose are called *dextro* or *D*-sugars, in distinction to *lævo* or *L*-sugars, which are derived from *lævo*-glycerose. *Dextro*-glucose may be written *D*-glucose or *d*-glucose, the latter is usual, but the former is preferable.



Since glycerose has only one asymmetric carbon atom, **C**, only two optical isomers, or epimers, are known. In higher sugars the number of asymmetric carbons increases, and the number of epimers increases, in accordance with van't Hoff's rule: $n = 2^c$; where n is the number of possible isomers, and c the number of asymmetric carbon atoms present in the compound. Thus, a hexose with four asymmetric carbons, has sixteen isomeric forms.

The H—C—OH group furthest from the aldehyde or ketone group in the sugar is the reference group. All D-sugars have a terminal configuration in this respect similar to *d*-glycerose.

Much confusion has arisen from the indiscriminate use of the small prefix *d* or *l* to denote chemical relationship as well as optical rotation, and to avoid mistakes it is now usual to employ the prefix (+) or (−) to indicate if a compound is dextro- or lævo-rotatory, the large capital D or L being used to denote lineal descent from the (+) or (−) chemical ancestor.

Thus, D (−) aldopentose is the five-carbon lævo-rotatory sugar derived from *d* (+) glycerose; L (+) ascorbic acid is the vitamin derived from *l* (−) glycerose. It will be noticed that the direction of optical rotation of the derivatives is not necessarily the same as that of the parent compound.

The close physical and chemical similarity existing between these pairs of optical isomers renders them indistinguishable in many of their reactions, but in the region of molecular dimensions found in the living cell, and ruled by the enzymes, the distinction between stereo-isomers becomes of primary importance in determining their susceptibility to attack. Carbohydrates and proteins represent the two great families of organic biological compounds constructed of optically active units.

Naturally Occurring Sugars

(A) MONOSACCHARIDES.

Sugar.	Sources.
(a) <i>Pentoses</i>	
$C_5H_{10}O_5$	
1. D-arabinose . . .	The glycoside <i>aloin</i> ; tubercle bacilli.
2. L-arabinose . . .	As the polymer <i>araban</i> in bran, husks, fruit skins; gum arabic, cherry and other gums; beet pulp; pectin;
3. D-ribose . . .	Yeast nucleic acid.
4. D-xylose . . .	As the polymer <i>xylan</i> in straw, husks, canes; and in wood gums.
5. L-xyloketose . . .	In pentosuric urines.

(A) MONOSACCHARIDES—*continued*.

Sugar.	Sources.
(b) ascorbic acid	
6. D-glucose .	As free sugar in fruits, flowers, honey ; as a glycoside in compound saccharides ; as a glycoside in many plants ; as a polymer in cellulose, starch, dextrin and glycogen.
7. D-fructose	As free sugar in fruits, flowers, honey ; as a glycoside in sucrose, raffinose and stachyose.
8. L-glucose .	In the glycoside <i>capsularin</i> .
9. D-galactose	As a glycoside in compound saccharides, including lactose ; in glyco- or galactolipides ; polymerised as galactan.
10. L-galactose	In flax seed mucilage.
11. D-mannose	As glycoside in compound saccharides ; as a glycoside in plants ; in muco- and other proteins ; as the polymer <i>mannan</i> in ivory nut.

THE MONOSACCHARIDES

Pentoses.— $C_5H_{10}O_5$.—These five-carbon sugars occur chiefly as pentosans and as glycosides. They are characteristic constituents of the nucleic acids of plants and animals, but never occur as free sugars, except in the pathological condition of **pentosuria**, when they are found in the blood and urine of animals.

The commonest pentoses are : *L-arabinose*, found in the arabans of gum arabic, cherry gum, and peach gum ; *D-xylose*, from the pentosans of straw, bran, and wood ; *D-ribose*, from nucleic acid.

The nature of the pentose excreted in urine is variable and depends on diet as well as on disease.

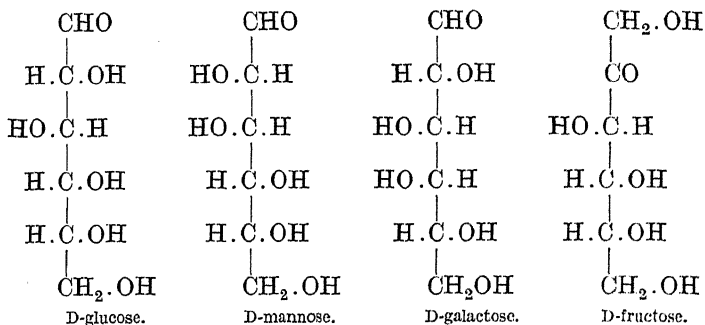
L-xyloketose is the commonest pentose found in pentosuria.

Pentoses may be obtained quantitatively by acid hydrolysis of the appropriate pentosan, and are detected by characteristic colour reactions (p. 112).

Hexoses.— $C_6H_{12}O_6$.—Out of twenty-four possible hexoses, only four are capable of utilisation by higher animals. These are the aldohexoses, *glucose*, *mannose*, and *galactose*, and the ketohexose *fructose*. All are D-sugars, being related structurally to *D-glycerose*.

The inter-relationship of these four isomers is shown in the so-called *projection formulæ* on p. 82.

D-Glucose, *grape sugar*, or *dextrose*, is widely distributed, both free and combined in glucosides, compound saccharides and polysaccharides. It occurs free in fruit and plant juices, in blood and



tissue fluids of animals, and in the alimentary tract as an end-product of carbohydrate digestion. Pure glucose is a white, crystalline solid. It melts at 100° C., losing one molecule of water of crystallisation at 110° C. Anhydrous glucose melts at 146° C. Further heating produces a brown mixture of decomposition products, termed *caramel*.

Glucose is very soluble in water, the solution being about one-third as sweet as sucrose of the same concentration. It is dextro-rotatory, $[\alpha]_D = +52.2^\circ$. Glucose is a typical aldose sugar in all its reactions. It reduces alkaline copper reagents, forms a characteristic osazone with phenylhydrazine, and is readily fermented by yeasts and bacteria.

D-Mannose is a relatively rare sugar, of slight importance in nutrition. It is obtained by acid hydrolysis of its natural polymer, *mannosan*, found most abundantly in Spruce sawdust and in the vegetable-ivory nut from the Tagua Palm. In concentrations of 0.5–4.0 per cent. mannose occurs in many higher proteins.

D-Galactose is obtained from the hydrolysis of lactose, its chief source; from raffinose; from the galactans of gums and mucilages; and from the galactolipides, or cerebrosides, of the central nervous system. It resembles glucose, but is much less sweet, and less soluble in water. It is dextro-rotatory, $[\alpha]_D = +80^\circ$. On partial oxidation, it forms the sparingly soluble *mucic acid*, by which it may be identified.

D-Fructose, *fruit sugar*, or *lævulose*, accompanies glucose in fruits, flowers, and their product, honey. It is obtained also from the hydrolysis of fructosides, the chief of which is sucrose, and from the polysaccharide, inulin. It crystallises with difficulty in fine, colourless needles, m.p. 110° C. Fructose differs from the other three fermentable hexoses in being a keto-sugar, or ketose, and in being lævo-rotatory, $[\alpha]_D = -92.0^\circ$. It is much sweeter and more reactive than glucose.

THE STRUCTURE OF THE SIMPLE SUGARS

Taking glucose as an example of the most familiar aldohexose, its structure and properties can be expressed by at least a dozen different formulæ. Each of these represents a stage in the history of sugar chemistry.

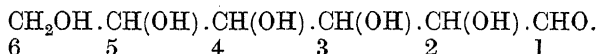
The Constitution of Glucose.—(1) $(\text{CH}_2\text{O})_n$, the *elementary formula*, found by combustion, shows that the substance has the C, H, and O, in the carbohydrate ratio.

(2) The *molecular formula*, $\text{C}_6\text{H}_{12}\text{O}_6$, calculated from the osmotic pressure, shows that the substance is of the hexose type. Mannose, galactose, and fructose have similar molecular formulæ.

(3) $\text{C}_6\text{H}_7(\text{OH})_5\text{O}$. Acetylation by acetyl chloride, $\text{CH}_3\text{CO}\cdot\text{Cl}$, gives a penta-acetyl derivative, showing that the original sugar contained five $-\text{OH}$ groups. Mannose, galactose, and fructose have similar formulæ.

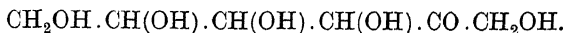
(4) The *aldose formula*, $\text{C}_5\text{H}_6(\text{OH})_5\cdot\text{CHO}$. Glucose contains one aldehyde, or $-\text{CHO}$, group, as shown by its oxidation to the corresponding carboxy acid, *gluconic acid*, $\text{C}_5\text{H}_6(\text{OH})_5\cdot\text{COOH}$, and also by its interaction with phenyl hydrazine to form a hydrazone. Mannose and galactose have similar aldose groups. Fructose does not contain an aldehyde group, and is represented by the *ketose formula*, $\text{C}_5\text{H}_7(\text{OH})_5:\text{CO}$, showing the ketone group: CO .

(5) The *linear formula*,



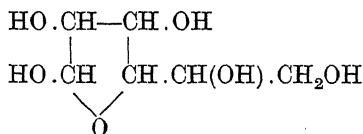
On complete reduction, glucose, mannose, and galactose are each converted into the same straight chain hydrocarbon, *hexane*, C_6H_{14} . This shows that the aldehyde group in the sugars must be terminal and not attached to some side-chain. Furthermore, the general stability of the sugars indicates that the five hydroxyl groups are attached to five different carbon atoms. Numbering to the left in accordance with chemical convention, the linear formula is 1-aldohexose: 1-aldohexose. This formula applies equally well to glucose, mannose, and galactose.

The linear formula for fructose is:—

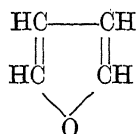


(6) *Projection Formulæ.*—The simple linear formula applies equally well to any of the aldohexoses, and to distinguish between these sugars the formula must be elaborated. This was done by Emil Fischer, who showed that the difference lay in the way in which the four secondary alcohol groups, $-\text{CH}(\text{OH})-$, were turned. By altering their arrangement on either side of a vertical

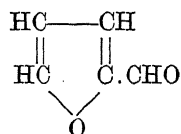
compounds, *furan* and *pyran*. The relationship is clearer when the sugars are written in cyclic form.



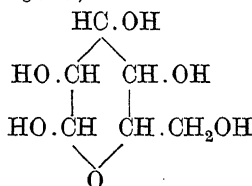
Glucofuranose
(1:4 ring form of glucose).



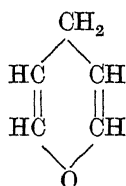
Furan ring.



Furfuraldehyde.



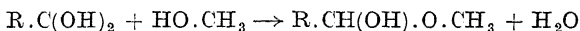
Glucopyranose.
(1:5-ring form of glucose)



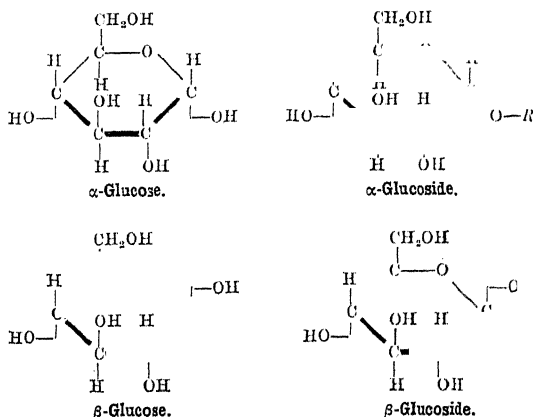
Pyran ring.

Furan is a 5-membered ring containing one oxygen atom, its aldehyde derivative, furfural, is obtained almost quantitatively when pentoses are distilled with strong acids. Higher sugars, such as hexoses, yield substituted furfurals under the same conditions. Pyran is a 6-membered ring containing one oxygen atom. The relative importance of these formulæ is under dispute. Haworth concludes that glucose occurs chiefly as the pyranose form. The furanose sugar being much more unstable, tends to revert to the pyranose form.

α - and β -glucose.—When glucose and other sugars are dissolved in methyl alcohol, and treated with HCl gas, monomethyl glycosides are obtained which no longer have the power of reducing alkaline copper solutions, showing that the aldenol group has been united to the methyl alcohol.



Each sugar, however, is able to yield two different glycosides, showing that some difference must exist between the two hydroxyls of the aldenol group. By careful hydrolysis, Fischer was able to recover two forms of glucose from these esters: α -glucose, of which the rotatory power is $+113^\circ$, and β -glucose, of which the rotatory power is $+19^\circ$. Ordinary glucose when freshly dissolved in water decreases in rotatory power until it reaches an equilibrium value of $+52.2^\circ$, showing that it is now a mixture of both α and β forms. The existence of these sugars is due to the fact that the terminal carbon 1, which is symmetric when in the form of the $-\text{CHO}$ group or the $-\text{C}(\text{OH})_2$ group, becomes asymmetrical directly one of the hydroxyls has condensed with a carbon 5 hydroxyl to produce the pyranose ring. The phenomenon of *mutarotation*, or change in rotatory power, and the existence of α - and β -glycosides is further proof that sugars normally exist in a ring structure. This is indicated in the modern *perspective* formulæ of the carbohydrates in which the plane of the ring is represented as being at right-angles to the plane of the paper, the various H and OH radicals being attached to perpendiculars.



(B) COMPOUND SACCHARIDES

Sugar.	Sources.	Components.
<i>(a) Dihexosides—</i>		
$C_{12}H_{22}O_{11}$		
Maltose (malt sugar)	Starch hydrolysis	glucose + glucose
Dextrinose (isomaltose)	Glycogen hydrolysis	
Lactose (milk sugar)	Beer, honey, liver	glucose + glucose
Cellobiose		
Gentiobiose	Milk of all mammals	galactose + glucose
Melibiose	Cellulose hydrolysis	glucose + glucose
Turanose	The glycoside amygdalin ;	glucose + glucose
Sucrose (saccharose, cane sugar)	the trisaccharide gentianose	
Trehalose	The trisaccharide raffinose	galactose + glucose
	The trisaccharide melizitose	glucose + fructose
	Fruits and sugar-storing plants, cane and beet	glucose + fructose
	In fungi ; as a polymer tre- halum in ergot and moulds	glucose + glucose
<i>(b) Trisaccharides</i>		
$C_{18}H_{32}O_{16}$		
Raffinose	Sugar beet, cotton seeds, molasses	galactose + glucose + fructose
Gentianose	Gentian root	glucose + glucose + fructose
Melezitose	In manna of Douglas fir, honeydew	glucose + glucose + fructose
<i>(c) Tetrasaccharide</i>		
$C_{24}H_{42}O_{21}$		
Stachyose	In leguminous seeds	glucose + glucose + galactose + fructose

THE COMPOUND SACCHARIDES

Compound saccharides are formed by condensation of two or more monosaccharides. Thus, a disaccharide contains two monosaccharides, a trisaccharide contains three, and a tetrasaccharide contains four.

Disaccharides.—Theoretically, any pair of monosaccharides may unite to form a disaccharide, but the term is commonly restricted to the *dihexose saccharides*, $C_{12}H_{22}O_{11}$, since they include the most familiar natural sugars, sucrose and lactose.

The general properties of a disaccharide depend on (i.) the constituent saccharides, and (ii.) the manner of linking. If the aldehyde or ketone groups are unaffected by the mode of union, the disaccharide is a reducing sugar, and, like all the monosaccharides, is able to react with alkaline copper solutions, such as those of Fehling and of Benedict. If, however, the aldehyde or the ketone groups of the constituent monosaccharides are immobilised by the linkage, the resulting disaccharide is a non-reducing sugar. This distinction is of considerable analytical importance.

A. Reducing Disaccharides (potentially active groups present).

MALTOSE, or *malt sugar*, a glucose-glucoside produced during the hydrolysis of starch and glycogen by acids or enzymes. It is the characteristic sugar of *malt*, or germinating barley, being formed by the breakdown of the reserve starch. It is very soluble in water, and is dextro-rotatory, $[\alpha]_D = +137.5^\circ$. The sugar is a typical aldose and gives a characteristic osazone. Maltose is easily hydrolysed by acids or the enzyme *maltase* into two molecules of glucose.

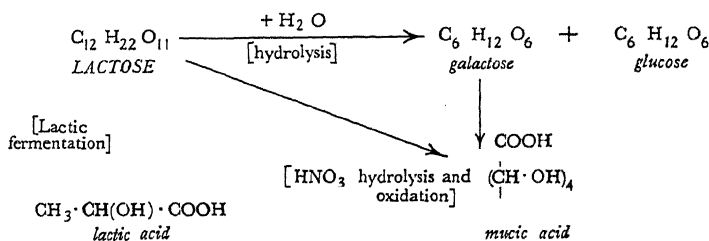
Since maltose is not attacked by the enzymes sucrase, lactase, or emulsin, all of which hydrolyse β -glucosides, it is inferred that it is an α -glucoside of α -glucose.

LACTOSE, or *milk sugar*, is found in the milk of all mammals. Its percentage ranges from about 2 in the rabbit, to about 7.5 in the elephant. Human milk has a lactose range of 5–7 per cent. Cow's milk contains 3.5–4.8 per cent. The sugar appears in the blood and the urine during lactation. It has not been detected conclusively in plants or in lower animals. Lactose is much less soluble and less sweet than the other common sugars, and is not fermented by ordinary yeasts. It is dextro-rotatory, $[\alpha]_D = +52.5^\circ$, has the properties of an aldose, and gives a characteristic osazone. Oxidation with nitric acid converts it, *via* galactose, into mucic acid, a sparingly soluble crystalline compound. This reaction serves to distinguish lactose, galactose, and their derivatives from all other sugars.

Lactose readily undergoes lactic fermentation by many organisms

found infecting milk, notably *Streptococcus lactis*. This leads to the formation of lactic acid, and the consequent "souring" of the liquid. Lactose is hydrolysed by a specific enzyme, *lactase*, found in the mammalian intestine, in emulsin (from almonds), and in some yeasts. It is not attacked by maltase, sucrase, or diastase, and is somewhat more resistant to acid hydrolysis than maltose or sucrose. The products of hydrolysis are the monosaccharides, glucose and galactose, showing that it is a galactoside, actually, 4- β -galactosido-glucose.

Reactions of Lactose.—



CELLOBIOSE, or cellulose, a β -glucose-glucoside obtained indirectly when the polysaccharide *cellulose* is acetylated and hydrolysed; the yield shows that at least one-third of cellulose is composed of cellobiose units. Cellobiose is hydrolysed by acids, by emulsin, lactase, and the specific enzyme, *cellobiase*, but not by maltase. The end-products are two molecules of glucose. In its properties cellobiose resembles maltose, but is less sweet and less soluble. It is not attacked by maltase, and hence is assumed to be a β -glucoside.

Maltose and cellobiose have now been shown to be stereo-isomers. Maltose is 4- α -glucosido-glucopyranose and cellobiose is 4- β -glucosido-glucopyranose.

Cellobiose has not been found to occur free in nature.

GENTIOBIOSE is found condensed with fructose in the trisaccharide *gentianose* from Gentian root. It is 6- β -glucosido-glucose. Gentiobiose is used in bacteriology to distinguish between closely related organisms, such as *Bacillus coli*, which does not attack it, and *Aerobacter aerogenes*, which does.

MELIBIOSE, a glucose- α -galactoside found condensed with fructose in the trisaccharide *raffinose* from beetroot. It is hydrolysed to glucose and galactose by acids, and by the enzyme *melibiase*, found in lower fermentation yeasts but not by "top-fermentation" yeasts. Consequently, the sugar is used to distinguish between these two yeast types. Melibiose is 6- α -galactosido-glucose.

B. Non-reducing disaccharides.

SUCROSE, *saccharose*, *cane sugar*, *beet sugar*, the most important industrial sugar, is widely distributed in plants, notably the stems of the sugar-cane and sugar millet, the root of the sugar-beet, and the trunks of some palms and maples. Sucrose crystallises readily as large, colourless monoclinic crystals, m.p. 160° C., which are soluble in water to the extent of 67 gm. per 100 gm. of solution at 20° C. The sugar is non-reducing, and does not show mutarotation in solution, which indicates the absence of potentially active aldehyde or ketone groups. It is readily hydrolysed by dilute acids, forming a molecule of glucose and one of fructose. Chemically, sucrose is an α -glucoside of fructose. Sucrose is dextro-rotatory, $[\alpha]_D = +66.5^{\circ}$, but after hydrolysis the resulting mixture of monosaccharides is lævo-rotatory (owing to the liberation of fructose), and this mixture is termed "invert sugar." Consequently, hydrolysis of sucrose is often described as *inversion*. The enzyme capable of causing it is called *invertase*, the systematic name being *sucrase*, which shows the particular sugar it attacks.

TREHALOSE, found in fungi and seaweeds, resembles sucrose generally, but is not hydrolysed by sucrase. By the action of acids or the enzyme *trehalase*, which accompanies it in fungi, it is converted into two molecules of glucose.

Trisaccharides.—Compound sugars formed by union of three monosaccharides. The most important are the *trihexosides*, $C_{18}H_{32}O_{16}$, of which four are known. Only one of them, mannotriose, is a reducing sugar.

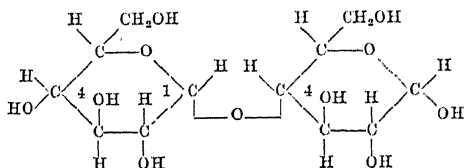
RAFFINOSE, the commonest trisaccharide, occurs in beetroot, cotton-seed (which contains 8 per cent.), and to a less extent in cereals and fungi. It is non-reducing, is dextro-rotatory, and closely resembles sucrose, m.p. 118.5° C. Structurally, raffinose is a fructo-gluco-galactoside, and liberates a molecule of each of these sugars on complete hydrolysis.

Trisaccharides have been detected in mammalian blood and tissue fluids, where they contribute to the "bound-sugar" fraction that only shows reducing properties after hydrolysis.

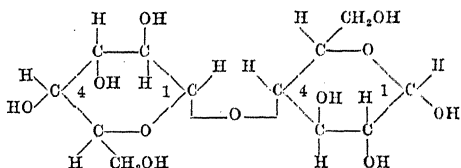
Tetrasaccharides.—A non-reducing tetrahexoside, stachyose, $C_{24}H_{42}O_{21}$, occurs in the seeds of leguminous plants. It is derived from glucose, fructose, and two molecules of galactose. On partial hydrolysis, glucose is split off, leaving the trisaccharide mannotriose.

The Structure of the Compound Saccharides.—The simplest formula for a disaccharide is $(C_6H_{11}O_5) \text{—} O \text{—} (C_6H_{11}O_5)$, showing the union of the two monosaccharide residues by an oxygen linkage, which is the point of hydrolytic attack. In the reducing disacchar-

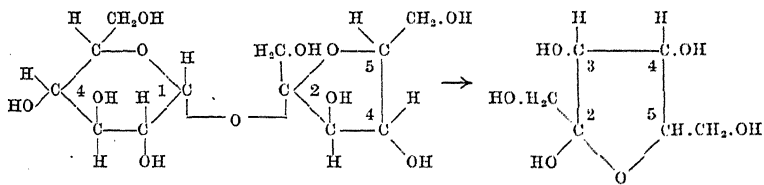
ides one of the residues, A, retains its aldehyde or ketonic properties as a reducing group. B, the other residue, by means of its own reducing group, is in glycoside union with A.



Maltose.
4- α -Glucosido- α -Glucose.



(Galactose residue)
Lactose.
4- β -Galactosido- α -Glucose.



(Fructose residue)
Sucrose.
2- α -Glucosido- α -Furanofructoside.

Fructose.
(Furanose form).

The glucose component in sucrose is in the α form, the fructose component is liberated on hydrolysis as fructofuranose. Hence the structure ascribed to sucrose is 2- α -glucosido- α -furanofructoside.

POLYHEXOSIDES, OR POLYSACCHARIDES

These carbohydrates differ profoundly from the sugars. Of high molecular weight, they do not form true solutions in water, but are either colloidal or insoluble. They have no sweet taste. They give none of the sugar reactions characteristic of aldose or ketose grouping.

Instead, many of them react chromatically with iodine, a property not possessed by the sugars. The carbohydrate nature of the

polysaccharides is shown by their conformity to the type formula, $C_m(H_2O)_n$, and by their cleavage into simple sugars on complete hydrolysis. Biologically, polysaccharides constitute the food reserve (starch), and structural material (cellulose, lignose, pentosan) of plants, and the easily mobilised carbohydrate reserve (glycogen) of animals. Substituted polysaccharides (heterosaccharides) are found as structural carbohydrate in the chitin of crustaceans and insects, and the pectin of fruits. All are classified chemically as *Holosaccharides* and *Heterosaccharides*.

HOLOSACCHARIDES.—Polysaccharides composed entirely of sugar units.

- (1) **Pentosans.**— $(C_5H_8O_4)_n$, polymers of a pentose unit. Chiefly *arabans* (from arabinose) and *xylans* (from xylose). Pentoses occur mostly in combination with other materials in vegetable gums and mucilages.
- (2) **Hexosans.**— $(C_6H_{10}O_5)_n$, polymers of a hexose unit. The most important members are the *glucosans*, or polymers of glucose, including cellulose, starch, glycogen, and their derivatives.

HEXOSANS

CELLULOSE, one of the chief organic products of vegetation. The cell-wall of all young plants is made of cellulose; in older cells other materials are incorporated, forming hemi-cellulose, ligno-cellulose, pecto-cellulose, and adipo-cellulose.

Industrial Sources of Cellulose

Cotton, flax	90–75 per cent. pure cellulose. Used for fine textiles.
Hemp, ramie, jute, manilla.	75–55 per cent. cellulose. Used for coarse textiles, wrapping, and cordage.
Wood-pulp, cereal straw, esparto.	40–20 per cent. cellulose. Used for paper-making and many other purposes.

Cellulose is obtained in quantity from cotton-wool. After being cleaned by alkaline washing, the product, either as filter-paper or cotton, is nearly pure cellulose. Low-grade paper is made from ligno-cellulose, which becomes friable and yellow on exposure to air and light.

Pure cellulose is a white, somewhat hygroscopic material, insoluble in water and common organic solvents, and relatively inert towards chemical reagents. It may be dissolved in : (a) ammoniacal cupric hydroxide (Schweitzer's reagent), (b) zinc chloride in hydrochloric

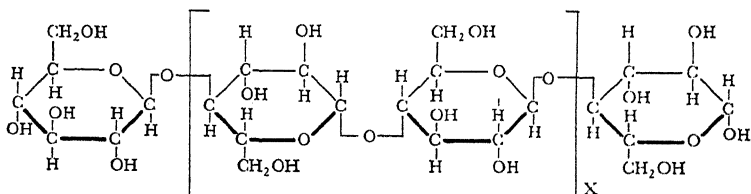
acid, (c) acetic anhydride, (d) sodium hydroxide and carbon disulphide, (e) concentrated sulphuric acid. In these reagents cellulose undergoes various changes giving rise to important industrial products, artificial silks, and plastics, such as "viscose," "cellophane." When dissolved in a mixture of nitric and sulphuric acid, cellulose forms the basis of gun-cotton, celluloid, collodion, and some types of artificial silk.

Cellulose is not acted on by the alimentary enzymes of higher animals, but is attacked by the widely distributed cytases, or cellulases found in germinating cereals, fungi, moulds, and bacteria.

The abundant cellulose in the diet of herbivora is digested by the flora found in the paunch and cæcum of ruminants, and the soluble carbohydrates liberated are absorbed by the host. Cellulose is not attacked in the human intestine. It is credited with the property of promoting peristalsis by mechanical stimulation, and forms part of the insoluble residue or "roughage" of the dietary.

On hydrolysis, cellulose yields glucose exclusively, and when acetylated and hydrolysed simultaneously (acetolysis) it yields 50 per cent. of cellobiose (4- β -glucosido-glucose), which indicates that cellulose is a chain of β -glucosidic units.

According to Haworth and his colleagues, cellulose has a molecular weight of the order of 30,000, and contains 100–200 glucose units.



Cellulose

M. Wt., 20,000–40,000.

Number of C_6 units, 100–200. $X = 80$ –100.

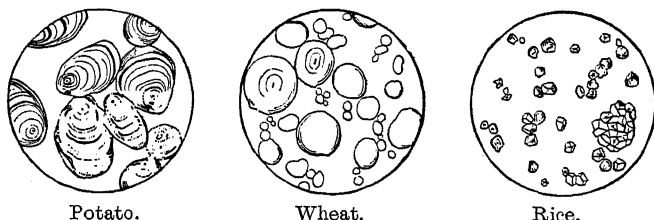
STARCH, or *Amylum*.—Starch is present in quantity in most vegetable foodstuffs, in cereals, and their products such as bread. It has not been found in the animal and it is probably restricted to organisms endowed with the power of photo-synthesis of carbohydrates. It is characterised by giving a deep blue colour with dilute iodine.

Starch is found in solid granules throughout plant tissues in leaves, stems, roots, fruits, and seeds. It is absent from a number of monocotyledons, notably the Snowdrop, Iris, and Hyacinth. The granules are usually made up of concentric layers formed round a hilum, suggesting that the material is deposited during cycles of

activity. When viewed by polarised light, a black cross is seen branching from the hilum.

The shape and size of the granule is characteristic of the plant, being ovoid and irregular in potato and arrowroot, oval in beans, discoid in wheat and barley, and polyhedral in rice and maize.

The starch granule is an aggregation of starch molecules which are resolved and rendered water-soluble by mild acid or enzyme hydrolysis, yielding "soluble starch."



Potato.

Wheat.

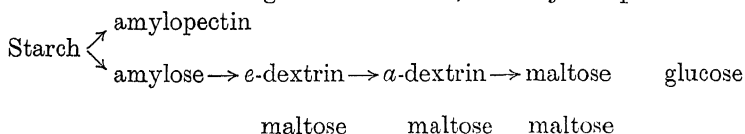
Rice.

FIG. 3.—Starch Grains ($\times 200$).

Average diameters in μ of common starches are: potato, 140–180; arrowroot, 155; haricot bean, 65; pea, 45–55; wheat, 45–55; maize, 30; oat (composite granules), 35–45; oat (single granules), 6–8; rice (single granules), 4–6.

Amylose and Amylopectin.—In 1858, Nägeli recognised two separate constituents of the starch granule, an external "starch cellulose" and an internal material, "granulose," the precursor of maltose. A separation was effected in 1906 by Maquenne and Roux who termed the inner material "amylose," and obtained an 80 per cent. yield. "Starch cellulose" they renamed "amylopectin." Amylose gives a blue iodine reaction, is soluble in water, and is completely hydrolysed to maltose by malt amylase. Amylopectin is insoluble in water, gelatinises on being boiled, and causes the gel formation in starch paste. Its colour reaction with iodine is variable, as different forms of amylopectin exist. Amylopectin is a phospho-carbohydrate, and before it is hydrolysed it must be dephosphatised. This may be accomplished by barley phosphatase. Subsequent treatment with malt diastase (amylase) converts the carbohydrate into a mixture of maltose, isomaltose, and glucose.

Products of Starch Hydrolysis.—Amylose is much more easily hydrolysed by acids and enzymes than cellulose. The process is both erosion and cleavage of the molecule, and may be represented:—



Saccharification is never complete. A residue of amylopectin, phosphates, and hemi-cellulose remains, its composition depending on the nature of the starch. Hydrolysis may be traced by means of the iodine test or by sugar estimation.

Waldschmidt-Leitz has obtained from malt two enzymes capable of hydrolysing starch in two different ways, as shown by the time at which the iodine blue colour is no longer given. α -amylase is a dextrinogen, and attacks the starch in the centrally placed glucoside linkages, producing dextrans. Under the attack of α -amylase, the blue reaction ceases to be given when about one-tenth of the starch has been converted into maltose, the rest being now a mixture of dextrans. Conversely, β -amylase is a saccharogen, and splits off maltose, unit by unit, from the ends of the starch molecule. Under its attack, the blue reaction persists until nearly all the starch is turned into maltose, the formation of dextrans being almost completely suppressed.

Dextrans are intermediate products of hydrolysis of starch and glycogen. They differ from starch in being soluble in cold water, and from sugars in being more readily precipitated by alcohol. The higher members, *e*-dextrans (erythro-dextrans), give red colours with iodine, the lower members, *a*-dextrans (achroo-dextrans), give no colour with iodine. All have a high dextro-rotation in solution, hence the group name, and all are completely hydrolysable to sugar. Purified by alcohol precipitation, they appear as white or yellow amorphous powers, readily soluble in water to form sticky liquids. The copper-reducing power depends on the method of preparation, and non-reducing dextrans have been obtained. Dextrans do not occur free in nature. Their presence imparts a characteristic flavour to bread crust, toast, and partly charred cereal foodstuffs.

DEXTRIN, or Starch Gum.—Industrial dextrin is made by heating dry potato starch to 210° C., or by autoclaving starch paste in presence of 1 per cent. citric acid. The product is chiefly *a*-dextrin, and is an important adhesive.

Maltodextrin is the general term applied to non-crystalline copper-reducing intermediate products of the action of diastase on starch.

GLYCOGEN, or animal starch, is found principally in liver and muscle, and also in other animal tissues, in all foetal tissues, in many fungi, but never in green plants. It is a rapidly mobilised carbohydrate, and is an essential constituent of the muscular machine. Glycogen closely resembles a higher dextrin. It is a white, water-soluble powder, $[\alpha]_D = +196.6^{\circ}$. It gives a mahogany red with iodine, and is precipitated from aqueous solution by addition of alcohol up to 60 per cent., or by saturation with solid ammonium

sulphate. It does not reduce alkaline copper solutions. Hydrolysis by acids, diastase or amylase, converts it first into lower dextrans, then maltose, and finally glucose. Glycogen may be distinguished from α -dextrin by its opalescence in solution, and by its precipitation by basic lead acetate.

Glycogen is relatively stable in hot 30 per cent. KOH or NaOH, and may be extracted by these reagents from fresh, finely minced tissues. It is purified by alcoholic reprecipitations, and by dialysis.

Glycogen Distribution in Animals.—Mammalian liver contains 3–7 per cent. of glycogen, or about a quarter to a half of the total reserve of the animal. The remainder is chiefly in muscle, which contains up to 1 per cent. Starvation rapidly lowers the liver glycogen value, but affects the muscle glycogen much less.

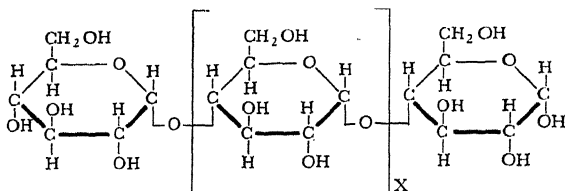
Structure of the Polyhexosides.—The molecular weights of these compounds is very high, and different values are obtained by different methods, which suggests that the carbohydrate is affected by the analytical treatment.

Thus, molecular weight determinations of acetylated starch give a minimal value of $(C_6H_{10}O_5)_{18}$ for the starch molecule, but this may have been lowered by depolymerisation during acetylation.

As regards composition, the pure polyhexosides are composed entirely of hexose units, most, if not all, of which are present in disaccharide form, as cellose in cellulose, and as maltose in starch and glycogen. These units are linked together to form long chains, the chains themselves possibly being united laterally by secondary attractions between neighbouring groups. Glycogen is a typical spherical colloid, cellulose is a linear colloid, and starch is intermediate in type.

The fact that the polysaccharides as a class have no sweet taste suggests that the secondary alcohol groups, $—CH.OH$, that confer sweetness and solubility on organic compounds, have been combined in some way during polymerisation of the sugar units. This polymerisation is different from condensation, such as occurs in the formation of ethers and esters, since polysaccharides, with the exception of lignose and cellulose, are easily depolymerised by dilute acids.

Although the individual molecule seems to be made up of some 10–30 glucose units, in aqueous solution the molecules aggregate to form polydispersed particles, or macro-molecules, which have molecular weights ranging from 60,000 to 300,000, the average being 200,000. From a study of the X-ray diagram of the crystal lattice of a typical polysaccharide (cellulose), Sponsler and Dore support the conclusion that the molecule is a chain of sugar units, each in pyranose form, united by α -linkages.



Starch and Glycogen

Starch : M. Wt., 5,000–200,000. Number of C_6 units ($= X$),
26–30.

Glycogen : M. Wt., 2,500. Number of C_6 units ($= X$), 12–18.

INULIN, or Dahlia starch, is the commonest example of a **fructosan**, or polymer of fructose. It is a reserve carbohydrate found in the roots and leaves of *Compositæ* and other plants. The chief sources are the tuber of the Dahlia, which contains 10 per cent., the Jerusalem Artichoke, Chicory, and Dandelion. It is not found in animals. Inulin is a white, tasteless powder, closely resembling starch, but giving no colour with iodine. Its solutions are lævo-rotatory, $[\alpha]_D = -40^\circ$, non-reducing, and not hydrolysable by amylases. When acted on by acids or the enzyme *inulase*, which accompanies it in the plant, inulin is converted completely into fructose.

Inulin forms a trimethyl derivative which yields trimethyl-fructofuranose on hydrolysis, showing that the polysaccharide is a chain of fructofuranose units. Irvine and Haworth believe the molecular weight to be about 5,000. Inulin has been used occasionally as a source of easily assimilated sugar in the treatment of diabetes mellitus, where glucose is not utilisable by the organism.

Levan is a synthetic polysaccharide of fructose formed by the action of *B. mesentericus* or *B. subtilis* on sucrose. It is structurally different from inulin in the linkage of the fructose units.

Levosin occurs in wheat flour, rye, bran and stubble. On hydrolysis it yields glucose and fructose in the proportions of 1 : 9.

HETEROSACCHARIDES. Polysaccharides containing carbohydrate and non-carbohydrate units. They are classified according to the sugar residues yielded on hydrolysis :

- (1) **Heteropentosans.**—Mucilages, gums, pectic substances, hemi-celluloses, all of which contain pentose units.
- (2) **Heterohexosans.**—Lignocellulose, pectocellulose, lipocellulose, all of which contain hexose units.
- (3) **Mucopolysaccharides.**—Hexosamine derivatives present in mucins and mucoids.

Holosaccharides

Name.	Class.	Formula.	Chief Source.
Araban . .	Pentosan.	$(C_5H_8O_4)_n$	Gum arabic.
Xylan . .	"	"	Straw, bran.
Cellulose . .	Glucosan.	$(C_6H_{10}O_5)_n$	Plant fibre.
Starch . .	"	"	Cereals, tubers.
Dextrin . .	"	"	Starch.
Glycogen . .	"	"	Liver, muscle.
Inulin . .	Fructosan.	"	Dahlia tubers.
Levan . .	"	"	Synthetic.
Levosin . .	Gluco-fructosan.	"	Cereals, bran.

Pectins, or vegetable mucilages, are widely distributed in plant tissues, roots, fruit pulp and rind, including the peel of apples, oranges and lemons. They are not extracted by cold water, but when boiled with weak acids, such as occur in fruits, or when attacked by the enzyme *pectase*, they are hydrolysed into substances which form characteristic jellies on cooling.

A typical pectin, such as that found in beetroot, yields (i) a polysaccharide, araban, and (ii) a complex acid, pectic acid, which is present as a calcium magnesium salt in the original pectin, and causes the gel formation.

Agar-agar, a mucilage from certain red seaweeds (*Rhodophyceæ*), contains 20–30 per cent. of galactan, a polymer of galactose, combined as an organic sulphate. Agar gels are superior to gelatin gels in bacteriological work. They do not melt at as low a temperature, and they are not attacked by proteoclastic micro-organisms.

Lignocellulose.—As plant tissues age, the cellulose of the stems becomes lignified, or converted into lignocellulose, the final form of which is **wood**. The change is due to combination between the cellulose and lignin (or lignon), which is a pentosan united to an aromatic residue, such as vanillin, *m*-methoxy-*p*-hydroxybenzaldehyde, $C_6H_3(OH)(OCH_3).CHO$.

Mucopolysaccharides

Natural mucins and mucoid secretions contain polysaccharides, either free or united to proteins as glycoproteins, and thus constitute a field common both to carbohydrate and protein chemistry. The polysaccharides are characterised by the presence of amino-sugar, or *hexosamine*, residues, and are classified by Meyer (1938) as (i) neutral mucopolysaccharides, (ii) mucopolysaccharides con-

taining uronic acid, and (iii) mucopolysaccharides containing both uronic and sulphuric acid residues.

Neutral mucopolysaccharides of known composition are represented by (i) chitin ; (ii) gastric polysaccharide, from gastric mucosa ; and (iii) bacterial polysaccharides.

Uronic sulphate-free mucopolysaccharides occur in vitreous humour, umbilical cord, synovial fluid, and certain bacteria.

Uronic sulphate-containing mucopolysaccharides are represented by *chondroitin sulphate*, in cartilage and other connective tissues ; *mucoitin sulphate*, from gastric mucin and from cornea ; *heparin*, the substance present in tissues that inhibits blood clotting.

Chitin forms the basis of the exo-skeleton of the *Insecta* and the *Crustacea*, and enters into the supporting tissue of fungi. It is a polymer of acetyl glucosamine, $\text{CH}_3\text{.CO.NH.C}_6\text{H}_{11}\text{O}_5$, with at least four units, and is the only prominent example of the use of a carbohydrate as skeletal material in heterotrophic organisms. Chitin is non-reducing and gives no colour with iodine. It is an insoluble and very stable carbohydrate, but may be hydrolysed by acids to an equimolecular mixture of glucosamine and acetic acid.

SACCHARIDE DERIVATIVES

(1) **Saccharide Alcohols**, or Mannitols.—Every simple sugar may be regarded as derived from a parent hydroxy alcohol, which can be obtained by reduction of the aldehyde or the ketone group of the sugar.

At least a dozen of these carbohydrate alcohols have been found widely distributed in plants, where they may represent a form of carbohydrate storage or a by-product in sugar metabolism. The most familiar are : *Erythrol*, $\text{C}_4\text{H}_6(\text{OH})_4$, found in algæ and mosses ; *D-Mannitol*, $\text{C}_6\text{H}_8(\text{OH})_6$, the alcohol corresponding to mannose, found widely distributed in fungi and manna from tree sap, especially that of the larch ; *D-Sorbitol*, an isomer of D-Mannitol, found in the fruit of the mountain ash and most *Rosaceæ*.

L-Sorbitol is used in one of the laboratory methods for synthesising the vitamin Ascorbic acid (p. 260). *Glycerol*, the trihydroxy alcohol corresponding to glycerose, is a characteristic constituent of oils and fats found in animals and plants.

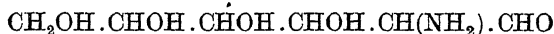
Chemically, the saccharide alcohols are stable, non-reducing substances, soluble in water and in alcohol, and possessing a very sweet taste. They are not fermented by yeasts, but are attacked by various moulds and bacteria, and are used in bacteriological work as a means of distinguishing between different organisms.

(2) **Saccharide Acids**.—These are formed by the oxidation of a

THE CARBOHYDRATES, OR SUGARS

terminal group in a monosaccharide. *D-glucuronic acid* is the most familiar, and is found as a detoxicant ester in urine after phenol administration. It occurs also in mucoproteins and a few hetero-saccharides. Its formula is given on p. 105. *L-Galacturonic acid* occurs in pectic acids.

(3) **Amino Saccharides.**—Sugars in which an OH group has been replaced by an amino group, -NH_2 . The natural amino saccharides are *hexosamines* of the general formula :



D-Glucosamine, or Chitosamine, obtained quantitatively by the hydrolysis of the polysaccharide *Chitin*, and as a final product of the hydrolysis of mucoitic acid from mucoproteins (p. 130). It is 2-amino glucose.

Chondrosamine, 2-amino galactose, is obtained by hydrolysis of chondroitic acid, a component of cartilage.

(4) **Saccharide Esters.**—Saccharides may form various esters or compounds connected by an oxygen linkage, —C—O—C— . When these esters are made up of two or more sugar molecules the product is a true sugar and belongs to the class of compound saccharides. When the ester is formed by the union of a sugar and a non-sugar or *aglucone* organic partner the product is a **glycoside**.

Glycosides comprise an important group of plant constituents, including the anthocyanin pigments that colour flower petals, the tannins, and many drugs, such as digitalis and strophanthin. Animal glycosides are represented by the cerebrosides and the nucleosides.

Esters are also formed by union of saccharides with inorganic acids, the hexose phosphates being of special interest since their formation precedes decomposition of the sugar molecule in metabolism.

THE HEXOSE PHOSPHATES

Hexose Diphosphate.—Fructofuranose-1 : 6-diphosphoric acid, (Harden-Young ester), $\text{C}_6\text{H}_{10}\text{O}_4(\text{H}_2\text{PO}_4)_2$, is formed in the first stage of the fermentation of glucose, mannose or fructose by yeast. The phosphate on hydrolysis by boiling yields fructose, and its formation is an example of the interconvertibility of the three monosaccharides. It is also an early product in the conversion of glycogen into lactic acid, in muscle.

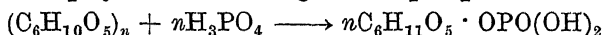
Fructose Monophosphate.—This ester has been obtained by Neuberg from the 1 : 6-diphosphate by partial hydrolysis. Unlike the diphosphate, it is fermented by yeast. Neuberg's ester is one of the two hexose phosphates found in muscle.

Glucose Monophosphates.—A crude monophosphate (Robison's ester) has been obtained by the action of yeast juice on glucose or fructose. It is a mixture of fructose monophosphate (Neuberg's ester) and glucopyranose-6-phosphoric acid (Embden's ester). The two esters have been separated by fractional crystallisation of their brucine salts. A glucopyranose-1-monophosphate (Cori's ester) is obtained by the action of phosphate on minced muscle.

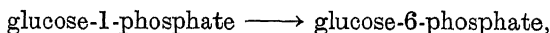
Pentose phosphates occur naturally in the nucleic acids, each of which is made up of four *mononucleotides*, or glycosides of pentose monophosphate (p. 130). 5-phosphoribose occurs in the nucleic acid of animal chromatin, and in the inosinic and adenylic acid of muscle (p. 291). 3-phosphoribose occurs in the guanylic acid, xanthylic acid and nucleic acid found in yeast (p. 348).

All these esters are variously described as sugar phosphates or phospho-sugars. In each of them only one of the three hydroxyl groups of phosphoric acid has been bound, and they are acidic compounds. The process by which they are formed is termed *phosphorylation* (p. 107).

Dialysed extracts of muscle, liver, brain and yeast contain an enzyme that phosphorylates each glucose unit in glycogen and thus disrupts the polysaccharide into glucose-1-phosphate.

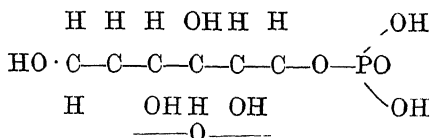


The reaction requires adenylic acid as a phosphate-carrier. Another enzyme in the extract catalyses the change:



the change being accelerated by Mg^{++} or Mn^{++} .

These transformations are of primary importance in carbohydrate metabolism (pp. 107, 292).

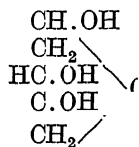


Glucose-1-phosphate (Cori ester).

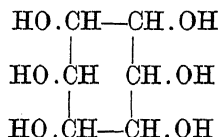
(5) **Glycals and Desoxy-sugars.**—By reduction and hydrolysis of substituted sugars it is possible to obtain unsaturated sugars, or *glycals*, in which two adjacent hydroxyl groups have been removed, leaving the linkage $—CH=CH—$. Glycals in presence of acid take up one molecule of water, thus restoring one of the hydroxyl groups, $—CH(OH)—CH_2—$, and forming a desoxy-sugar, which has one oxygen atom less than the parent monosaccharide. One of these sugars, 2-desoxy-D-ribose, occurs naturally in animal nucleic acid,

while several ω -desoxy sugars (methyl sugars), in which the ω -, or terminal $-\text{CH}_2.\text{OH}$ group has become $-\text{CH}_3$, are widely distributed among plants.

Examples of ω -desoxy sugars are: 3-desoxyglyceric aldehyde, or lactic aldehyde, $\text{CH}_3.\text{CH}(\text{OH}).\text{CHO}$, found in poplar leaves; 6-desoxy-D-galactose, found as a polymer in Japanese seaweed and in gum tragacanth; 6-desoxy-D-allose, or digitalose, found in the glycoside digitalin; and 6-desoxy-L-mannose, or L-rhamnose, found in many glycosides.



2-desoxy-D-ribose.



Inositol.

CYCLOSES or CYCLOHEXANOLS

These compounds are isomeric with the saccharides, since they have the general formula $\text{C}_m(\text{H}_2\text{O})_n$; they are, however, chemically distinct from the sugars, being hydroxy derivatives of benzene in which there is no potential aldehyde or ketone group. On account of their hydroxylation, cycloses are very soluble in water and have a sweet taste. As a class they are stable, non-reducing compounds, and do not give the general tests for carbohydrates. Seven cycloses are known to exist.

Inositol, $\text{C}_6\text{H}_6(\text{OH})_6$, hexahydroxy-hexahydrobenzene, was formerly called "muscle sugar." It is widely distributed throughout the plant kingdom, especially in the form of **phytol**, or inositol hexaphosphate, which occurs as an insoluble calcium magnesium salt, and provides a phosphate reserve for the plant. Inositol is found in nearly all organs of the animal, especially muscle and glands. It is absent from the urine, except in the rare and obscure condition of inositoluria.

CHAPTER VII

REACTIONS OF CARBOHYDRATES

NATURAL carbohydrates are stable compounds under conditions of neutrality. Changes of reaction in the direction of acidity or alkalinity untabilise them in various ways, depending on the class to which they belong. The changes undergone include :—

- (1) Decomposition by alkalies.
- (2) Decomposition by strong acids.
- (3) Hydrolysis by weak acids.
- (4) Reduction.
- (5) Oxidation.
- (6) Hydrazone and osazone formation.
- (7) Esterification, and phosphorylation.

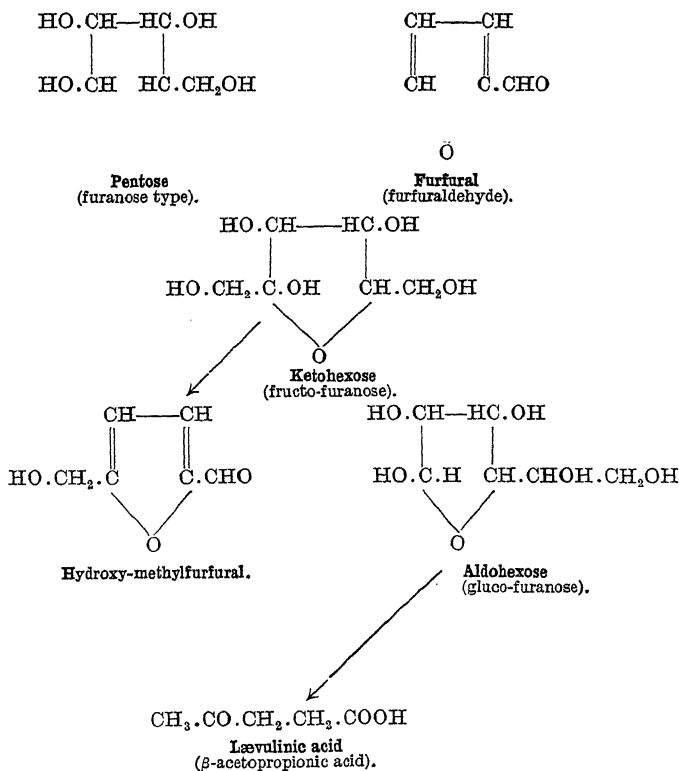
(1) **Decomposition by Alkalies.**—Polysaccharides and non-reducing saccharides (sucrose) are relatively stable in presence of alkalies, but reducing sugars are very unstable, and readily undergo three types of change : (a) non-oxidative molecular rearrangement, (b) non-oxidative molecular fission, and (c) oxidation and fragmentation. The type of the change depends on temperature, concentration of alkali, and presence of oxidising agents. Changes of the first and second type have been studied by Lobry de Bruyn and by Nef, and are extremely complicated. Changes of the third type occur when sugars are aerated in alkaline solution, the chief products being simple organic acids (lactic, pyruvic, propionic, acetic, glyoxylic, oxalic, and, finally, carbonic acid).

Heated in alkaline solution along with an oxidising agent, such as a cupric, ferric, or mercuric salt, all monosaccharides and all compound saccharides containing aldehyde groups are rapidly oxidised to acids containing the same number of carbon atoms. At the same time, the metal is reduced in proportion to the amount of the sugar present, and this reaction is made the basis of the principal methods for detecting and estimating reducing carbohydrates.

(2) **Decomposition by Strong Acids.**—All carbohydrates higher than tetroses on being boiled with excess of strong acids (HCl or H_3PO_4 are best) evolve *furfurals*, which may be detected by colour

tests and separated by distillation. Furfural liberation is important as indication of a furanose pattern latent in the original carbohydrate. The type of furfural depends on the sugar residue present. Pentoses are quantitatively converted into simple furfuraldehyde on distillation with 20 per cent. HCl. Ketohexoses yield about 20 per cent. substituted furfural; and aldohexoses liberated about 1 per cent. of hydroxy methylfurfural. These differences in reaction are used to distinguish pentoses, ketohexoses, and aldohexoses. Polysaccharides react in accordance with the nature of the saccharide units they contain.

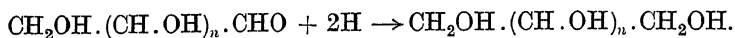
Lævulinic acid is the chief product of the action of concentrated acids on hexoses and hexosides. It may be regarded as arising by the opening up of the furan ring.



(3) **Hydrolysis by Weak Acids.**—Compound saccharides and many polysaccharides (starch, glycogen, inulin) are readily hydrolysed to simple saccharides by boiling with weak acids. The simple

saccharides themselves are stable in weak acid solutions unless oxidising agents are present, when they are converted into organic acids, although much less rapidly than when they are in alkaline solutions.

(4) **Reduction.**—Reduction of an aldose produces the parent saccharide alcohol :—

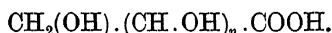


Reduction of a ketose produces a mixture of two isomeric hydroxy alcohols.

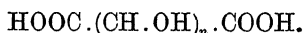
Saccharide alcohols closely resemble the original sugars in solubility, sweetness, optical activity, and isomerism. Once formed, they are very resistant to further reduction, and require drastic reagents, such as boiling HI, to reduce them to the parent hydrocarbon.

(5) **Oxidation.**—Aldoses may undergo oxidation in three different ways to produce acids having the same number of C atoms as the parent sugar.

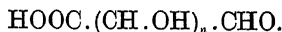
(i) Oxidation of the aldehyde group alone. This forms the corresponding *saccharinic acid* :



(ii) Oxidation of the terminal alcohol group as well as the aldehyde group. This produces the corresponding dicarboxy, or *saccharic*, acid :

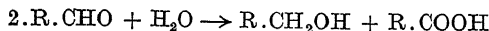


(iii) Oxidation of the terminal alcohol group alone. This can only occur if the aldehyde group is protected during the oxidation. The resulting compound is a *uronic acid* :



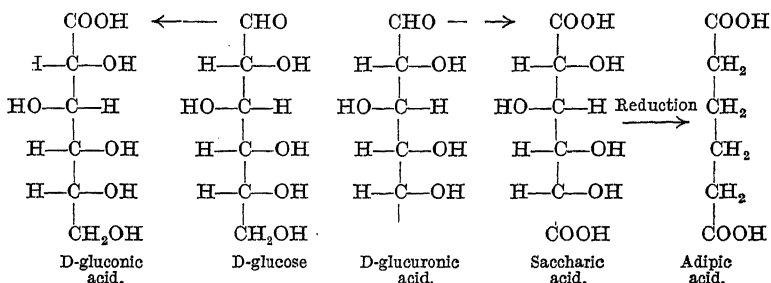
For example, D-glucose on oxidation with bromine water forms the saccharinic acid, D-gluconic acid. On oxidation with nitric acid, D-glucose forms saccharic acid. To obtain the aldehyde-acid, glucuronic (or glyceuronic) acid, the glucose must be oxidised in the form of a glucoside, in which the aldehyde group is combined, and so escapes oxidation.

The Cannizzaro reaction.—In alkaline solution, aldehydes tend to undergo an oxidation-reduction transformation with production of the corresponding alcohol and acid, which is neutralised by the alkali.



In concentrated alkali, and in the absence of an oxidiser, aldehyde sugars by this transformation give rise to saccharinic acids.

Saccharide Acids



D-glucuronic acid is the most important of the uronic acids. It is excreted in the urine as a glucoside after administration of higher alcohols, phenols, terpenes, chloral, and similar compounds. This represents a form of *detoxication* in which the foreign substance is rendered inert by being condensed with the aldehyde group of glucose. The resulting glucoside is partially oxidised to form the uronic compound.

Saccharic acid, tetrahydroxy adipic acid, forms a sparingly soluble acid potassium salt that is useful in identifying glucose and glucosides.

Mucic acid, an isomer of saccharic acid, is formed by oxidation of galactose and galactosides by means of warm nitric acid. It is almost insoluble in cold water, and provides an important means of identifying galactose.

The ketonic sugars are resistant to bromine oxidation, and thus may be separated from the aldoses. On more powerful oxidation, ketoses rupture at the carbonyl group, with formation of two acids.

Oxidation of compound saccharides is usually accompanied by hydrolysis unless special precautions are taken. Polysaccharides and non-reducing saccharides are relatively resistant to oxidation.

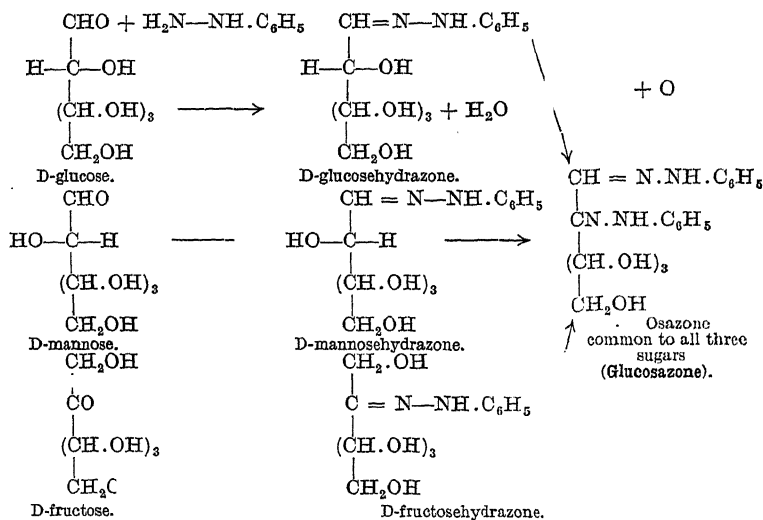
A reducing disaccharide on oxidation yields a monocarboxy *bionic* acid, the $-\text{CHO}$ becoming $-\text{COOH}$. These acids, like all hydroxy acids with hydroxyl in the 3, 4, 5, or 6 position, readily close up to form a *lactone*, or ring compound made by condensation between $-\text{COOH}$ and one of the $-\text{OH}$ groups of the same carbon chain.

(6) **Hydrazone and Osazone Formation.**—Aldoses and ketoses react with hydrazines, or compounds of the type $\text{R.NH}-\text{NH}_2$. This involves replacement of the aldehydic or ketonic: O by the group $\text{R.NH}-\text{N}=\text{}$, the resulting product being called a hydrazone. Thus, by means of the reagent **phenylhydrazine**, $\text{C}_6\text{H}_5.\text{NH}-\text{NH}_2$, phenylhydrazones can be obtained from all the reducing sugars. These hydrazones, with the exception of mannose phenylhydrazone,

are freely soluble in water, and can be reconverted into the sugar by treatment with benzaldehyde.

However, when the sugar phenylhydrazones are boiled with excess of phenylhydrazine an important reaction occurs. The alcohol group adjacent to the hydrazone radicle is oxidised to a ketone or an aldehyde group, which in turn reacts with phenylhydrazine to produce a double hydrazone, or **osazone**. Sugar osazones are sparingly soluble in water, have characteristic crystalline shapes and melting points, and provide a valuable means for sugar identification, alone or in mixtures.

Furthermore, D-glucose, D-mannose, and D-fructose yield the same osazone, showing that their molecules contain a similar arrangement of carbon atoms once the individuality of the C_1 and C_2 groupings has been lost by union with the hydrazine.



(7) **Esterification.**—Sugars can unite by means of their hydroxyl groups with acids, ketones, and other compounds to give a variety of esters, some of which occur naturally in the organism, while others have been of use in determining the constitution of the carbohydrates.

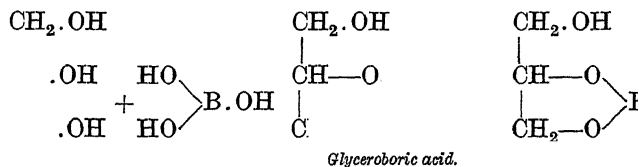
The formation of penta-acetates is evidence that hexoses only contain five free hydroxyl groups, the sixth oxygen being present in cyclic linkage. The discovery of two different methyl glucosides led in turn to the discovery of the α - and β -sugars.

With acetone, sugars give crystalline mono- and di-acetone derivatives, formed by union of the acetone: CO group with two adjacent —OH

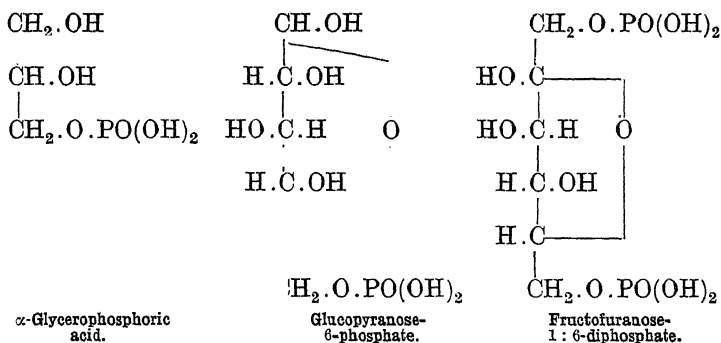
groups which must be in the *cis* position, or on the same side of the —C—C— axis. Since in these esters two adjacent carbons are held in the crab-like grip of a third —O—C—O— group they are termed *chelate* compounds. By previous formation of these acetone derivatives, pairs of *cis* hydroxyl groups in a sugar may be masked or protected during subsequent chemical manipulations, as, for example, those employed in the synthesis of ascorbic acid (vitamin C) from L-sorbose.

Sugars, glycerol and other polyhydroxy compounds also unite with the hydroxyl groups of weak acids to form esters that have the characteristic of being more acidic than the parent acid.

For example, boric acid, H_3BO_3 , is a very weak acid, and occurs in neutral solution largely as non-ionised $(\text{HO})_3\text{B}$. On addition of boric acid or a neutral borate to glycerol, esterification rapidly takes place at ordinary temperatures to produce a monoborate, which now displays acid properties and may be estimated by alkaline titration.



Phosphoric acid, H_3PO_4 , which occurs in solution as the ion $(\text{HO})_2\text{PO.O—}$, combines by means of one of its unionised hydroxyls to form glycerophosphoric and hexose phosphoric esters, which are of great biological importance (p. 100).



(8) **Rotation of Polarised Light.**—By use of the polarimeter, all carbohydrates can be divided into a dextro-rotatory class and a lævo-rotatory class. The sugar D-fructose is the commonest lævo-rotatory saccharide, and for this reason is termed *lævulose*. Each pure saccharide has a specific rotatory constant, which is determined under equilibrium conditions so as to avoid confusion due to mutarotation.

Specific Rotation.—This is defined as the rotation in angular degrees produced by a length of 1 decimetre of solution containing 1 gm. of solute in 1 ml. It may be expressed: $[\alpha] = \frac{100a}{lc}$, in

which $[\alpha]$ = specific rotation ;

a = observed angular rotation ;

l = length of the column of solution, in decimetres ;

c = concentration, in grams per 100 ml. of solution.

The value of the specific rotation depends on the temperature, which is fixed for reference purposes at 20° C. It also depends on the nature of the light source employed. The bright yellow D lines of the sodium spectrum or the yellow-green mercury line, 5461 Å, are the usual illuminants. The light used is indicated by affixing D or Hg to the symbol denoting the specific rotation.¹

Sugar in Aqueous Solution.	$[\alpha]_D$	Sugar in Aqueous Solution.	$[\alpha]_D$
L-xylose .	+ 19.0	D-fructose	— 92.0
D-arabinose	—105.0	Lactose .	+ 52.5
L-rhamnose	+ 8.9	Maltose .	+137.5
D-glucose .	+ 52.2	Sucrose .	+ 66.5
D-galactose	+ 80.5	Invert sugar	— 20.6
D-mannose	+ 14.6	Raffinose .	+105.2

ANALYTICAL REACTIONS OF CARBOHYDRATES

(1) General Test for Carbohydrates. The Thymol Test

To a small quantity of the solid or the solution (0.5 ml.) add 3 drops of 3 per cent. thymol in alcohol, 5 ml. of strong HCl, and about 2 gm. of solid NaCl, or enough to fill 1 cm. of the test tube. Boil *carefully* over a small flame, for 1–3 minutes.

A carmine colour is given by carbohydrates.

The NaCl is added to make the mixture boil quietly.

(a) The test may be applied equally well to insoluble carbohydrates, such as cellulose or wood, as the process of boiling with the acid brings about their solution.

(b) The pigment is due to condensation between furfural liberated from the carbohydrate and the thymol. It may be extracted by amyl alcohol, or by chloroform.

¹ The technique of polarimetry depends very much on the types of instrument in use, details of which will be found in the various practical text-books by Cole, Hawk and Bergeim, and Plimmer. Improvements and modifications in optical saccharimetry are described in "Recent Advances in Analytical Chemistry," Part II., by Ainsworth Mitchell.

(c) Many other phenols may be used instead of thymol, such as α -naphthol (Molisch's original form of the test), resorcinol, etc.

(d) Higher proteins give the reaction owing to the presence of glucose residues present as hexosamines.

(e) The reaction cannot be applied to the detection of sugar in urine because the indoxyl present forms a pigment of its own with the phenol.

(f) Oxidation is necessary for the development of the colour.

(2) Group Test for Polysaccharides. The Iodine Test

To 5 ml. of the solution, which must not be alkaline, add a drop of 1 per cent. iodine. Note the colour produced (p. 93).

Deep blue.

Red-brown.

No change, other than pale yellow due to iodine.

Starch.

Glycogen.
Dextrin.

Polysaccharides absent.¹

Distinguish between glycogen and dextrin by saturating the original solution with solid ammonium sulphate. Glycogen is completely precipitated; dextrin is not. Filter. Test the filtrate with iodine, a red-brown colour indicates dextrin.

(3) Group Test for Saccharides. The Nitro-chromic reaction

To 3 ml. of the solution add about 5 ml. of concentrated nitric acid and 5 drops of 5 per cent. potassium chromate. Mix well. A blue colour develops in about a minute if sugar be present.

(a) The test depends on the presence of $-\text{CH.OH}$ groups, and therefore is given by all primary and secondary alcohols, including glycerol, and also by formaldehyde, lactic acid, hydroxy-butyric acid, and mandelic acid.

(b) Polysaccharides free from sugars give no colour with the test until they have been hydrolysed by the action of the nitric acid. This may require some hours in the cold solution.

(c) The test is negative with proteins, fats, and the normal constituents of urine.

Moore's Test.—To 5 c.c. of solution add 10 drops of 20 per cent. sodium or potassium hydroxide. Warm gently. All the reducing sugars cause a colour change in the solution, passing from pale yellow to dark brown.

¹ Neither inulin, a polysaccharide of fructose, nor achroo-dextrin gives a colour with iodine. The first is detected by hydrolysis, when it only liberates fructose (distinction from the non-reducing sugar, sucrose), the second is rarely met with free from erythro-dextrin.

(a) The test is an example of the Nef transformation undergone by aldoses and ketoses in alkaline solution. It is of not much analytical value, but serves as a basis for the "reduction tests."

(b) Polysaccharides and non-reducing sugars do not react.

(c) The colour is due to "resinification" or polymerisation of the aldehydes liberated by the action of the alkali on the sugar.

Special Tests for Saccharides. The Copper-reduction Tests

(1) **Trommer's Test.**—To 5 ml. of solution add 2 drops of 5 per cent. copper sulphate and 10 drops of 20 per cent. sodium hydroxide. A light blue precipitate of copper hydroxide forms and may dissolve to form a blue solution if sufficient saccharide be present. Boil carefully. The blue changes to orange-red (cuprous oxide) if a reducing sugar be present. In the absence of reduction, the mixture turns black on prolonged boiling; for this reason excess of copper must be avoided, as the black colouration (cupric oxide) may obscure a slight red reaction.

(2) **Fehling's Test.**—Mix equal parts of Fehling's reagent, A and B, about 2 ml. of each. A deep blue solution is formed. Boil for about a minute. No change is observed. Add an equal volume of the solution to be tested, and boil again. An orange-red precipitate forms if a reducing sugar be present.

Benedict's Qualitative Test.—Add 8 drops of the solution to 5 ml. of Benedict's qualitative reagent. Boil for one and a half minutes, or, preferably, place in boiling water for two to three minutes. Remove, and allow to cool. A green turbidity with a yellow precipitate indicates 0.1–0.3 per cent. reducing sugar in the original solution. A dense orange precipitate with a clear supernatant fluid indicates more than 1.5 per cent. of sugar.

(a) The reagent is designed specifically for the detection of sugar in urine. It consists of: 17.3 gm. crystalline $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, with 173 gm. Na citrate, and 100 gm. anhydrous Na_2CO_3 in 1 litre water.

(b) Benedict's qualitative reagent must not be confused with his quantitative reagent, which is used only for sugar estimation.

(3) **The Methylene Blue Test.**—Make 5 ml. of the solution alkaline with a few drops of 20 per cent. sodium hydroxide. Add a few drops of 0.1 per cent. methylene blue. Boil. The blue colour of the solution is discharged rapidly if a reducing sugar be present, but returns temporarily on aerating the contents of the tube by shaking.

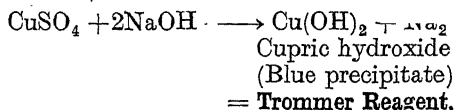
(a) The test is not specific, and is almost too delicate to be of general application. The solution rapidly regains its colour on aeration owing to reoxidation of the dye precursor, leuco-methylene blue.

(b) The reaction is important in providing a useful "internal indicator" for copper reduction tests, such as that of Fehling, and simplifies the determination of an end-point in an estimation.

The above special tests are given by all monosaccharides and most of the compound saccharides, which in consequence are grouped as the **reducing sugars**. The commonest reducing sugars are lactose, maltose, glucose, fructose, galactose, mannose, and the pentoses; while sucrose is the only common non-reducing sugar.

Hydrolysis Test for Sucrose.—Boil 5 ml. sucrose solution with about 2 ml. mixed Fehling reagent. There is no reduction. Acidify the warm mixture with a few drops of concentrated hydrochloric acid till the colour changes to light green. Boil again for a minute. The acid hydrolyses the sucrose to glucose and fructose, both of which are reducing sugars. Add sufficient alkali (20 per cent. NaOH) to regenerate the Fehling reagent, as shown by the return of the original blue colour. Reduction now occurs if the solution be warm.

MECHANISM OF THE COPPER REDUCTION TESTS

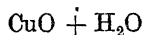


Dissolved by
tartrate
= **Fehling Reagent**

Dissolved by
citrate
= **Benedict Qualitative Reagent**

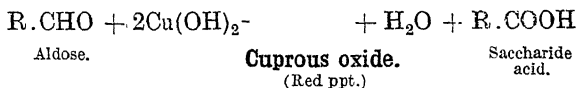
On heating alone

Unchanged by heating alone



Cupric Oxide
(Black ppt.)

Heated with a reducing sugar



Notes on the Reagents.—(a) Trommer's reagent is unstable and must be prepared during the process of carrying out the test, which is delicate and reliable if properly applied.

(b) Fehling's reagent A is 34.65 gm. crystalline $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 500 ml. water. Reagent B is 60 gm. NaOH and 173 gm. NaK tartrate (Rochelle Salt) in 500 ml. water.

(c) The reagents are kept separate owing to the tendency of the mixture to become unstable and undergo spontaneous reduction when boiled, even in the absence of a reducing sugar.

(d) The tartrate is added to keep the copper hydroxide in solution and prevent the formation of a black precipitate of cupric oxide during boiling.

(e) When testing for traces of sugar it is advisable to dilute the mixed Fehling reagent 1:5, otherwise the deep blue may conceal a faint precipitate of cuprous oxide.

(f) When applied to urine the sensitivity of the test is lessened by the presence of ammonia and creatinine, both of which dissolve cuprous oxide.

(g) If KSCN and $K_4Fe(CN)_6$ be present, as in *Benedict's quantitative reagent*, the red cuprous oxide does not form, being replaced by a white precipitate of cuprous thiocyanate. Hence, the end-point of the reaction is white.

(4) **The Ketose Test.**—To 3 ml. of sugar solution add about 2 ml. of concentrated hydrochloric acid. Boil carefully. An orange colour is given by sucrose and by its constituent saccharide, fructose. Since both these sugars contain a ketone group, $C=CO=C$; they are called ketoses. Fructose is the only common reducing sugar that gives this test.

(5) **The Furfural Test.**—Mix 5 c.c. of sugar solution with an equal volume of concentrated hydrochloric acid and 5 drops of 5 per cent. resorcinol in alcohol. Warm gently in a water-bath. A red colour is given at about $65^\circ C$. by pentoses, ketoses, and compound saccharides that liberate these sugars on hydrolysis. Aldoses only react in strong solution, and on prolonged boiling.

(a) This reaction, known as Seliwanoff's test, will distinguish fructose from all the other reducing sugars, and will distinguish sucrose from the other disaccharides.

(b) The reaction depends on the fact that aldoses do not liberate furfurals when boiled with the diluted hydrochloric acid, so if the test is being applied to a solid sugar it is always necessary to dilute the acid with an equal volume of water, otherwise the test is only a variant of the original general test for carbohydrates.

(c) Oxidation is necessary for the development of the colour, and the best results are obtained by using hydrochloric acid that has gone yellow owing to formation of chlorine peroxide following exposure to light, or has been activated by addition of 1 per cent. $FeCl_3$ or H_2O_2 .

(d) By substituting other reactants for the resorcinol, it is possible to distinguish between pentoses, ketoses, and aldoses. Thus, using orcinol (Bial's test), pentose gives violet-blue; methylpentose and ketose turn orange-red; aldose does not react. Naphtho-resorcinol under similar conditions is used to detect glycuronic acid in urine and to distinguish it from true sugars, as the pigment obtained from a uronic acid differs from the pigment obtained from a sugar in being ether-soluble.

SUMMARY OF THE FURFURAL REACTIONS FOR SUGARS

HCl.	Phenol, etc.	Pentose.	Ketose.	Aldohexose.	Glycuronic Acid.
35%	None	—	yellow	—	—
35%	Thymol	purple	carmine	carmine	carmine
35%	α -naphthol	violet	purple	purple	purple
35%	Resorcinol	carmine	orange	orange	carmine
35%	Orcinol	blue	carmine	carmine	carmine
15%	Resorcinol	carmine	orange	—	carmine
15%	Orcinol	violet	orange	—	carmine
15%	Naphtho- resorcinol	purple	purple	—	purple, soluble in ether.

It is important to note that a fully concentrated aqueous solution of hydrochloric acid contains about 30–35 per cent. HCl. Hence, in carrying out tests requiring this concentration, the reagents and the sugar should be added in solid form or dissolved in a minimum amount of solvent, and excess of concentrated acid added. Acid of 15 per cent. concentration is obtained, approximately, by adding an equal volume of concentrated acid to the sugar solution. Excess of the phenol should be avoided, and heating is best done in a boiling water-bath.

Fermentation by Yeast.—The yeast organism decomposes fermentable sugars into alcohol and carbon dioxide in accordance with the general equation :



(a) *Acid Formation.*—Shake up about 25–30 ml. of sugar solution with 2–3 gm. of baker's yeast (*Saccharomyces cerevisæ*). Transfer about 5 ml. into a test tube, and add a few drops of the indicator phenol red. The reaction of the mixture is acid (yellow). Add a drop or two of N/10 NaOH until the mixture is just alkaline (red), and incubate the tube at 45°–50° C. for a few minutes. The contents gradually become acid owing to liberation of carbon dioxide from the fermenting sugar. On neutralising again, the process is repeated, and continues until all the sugar is fermented. After the fermentation has continued for about an hour test the contents of the acid mixture with iodine diluted until it is almost colourless. A dark brown colour indicates the presence of glycogen in the yeast.

Ordinary compressed yeast usually gives little or no colour with iodine as the organism is in a state of starvation, having exhausted its glycogen reserve.

(b) *Alcohol Production*.—Fill the closed limb of a fermentation tube with the yeast-sugar mixture so as to leave no air bubbles. Place tube in an incubator at 40–50° for 1 hour, or leave for 24 hours at room temperature. If a fermentable sugar be present it is broken down into alcohol and carbon dioxide, which collects as a gas in the closed limb of the tube.

1. *Detection of carbon dioxide*.—Add 2–3 ml. of 20 per cent. sodium hydroxide to the tube; close the orifice, and invert the tube so as to mix the contents. The gas is rapidly absorbed by the alkali, and a negative pressure develops in the tube.

2. *Detection of alcohol*.—Unseal the tube. Add about 10 drops of an aqueous iodine solution, and mix by shaking. The iodine is decolourised by the alkali, and also interacts with any alcohol present to produce iodoform, which is recognisable by its smell.

Fermentable Sugars.—Four hexoses only are fermented by ordinary yeast, namely: *glucose*, *mannose*, *galactose*, and *fructose*. Of the commoner disaccharides, *maltose* and *sucrose* are attacked while *lactose* is not. Pentoses as a class are non-fermentable by yeast.

The test thus serves to distinguish pentoses and lactose from all other common reducing sugars. It is frequently applied to urine to distinguish *glucose* from (1) *lactose*, (2) *urinary pentose*, and (3) *glycuronic acid*, all of which reduce alkaline copper solutions.

Oxidation Tests.—Galactose and its derivatives (lactose, and galactosides) on oxidation by hot nitric acid yield mucic acid which is almost insoluble in cold water. Other saccharides yield saccharic acid which is much more soluble, but forms an identifiable acid potassium salt. The following test, which must be carried out in a fume chamber, will detect both galactose and glucose, or sugars giving rise to them. It is not applicable to dilute solutions, which must be concentrated to syrups before oxidation.

(a) *Mucic acid*.—Add 10 ml. of strong nitric acid to about 2 gm. of lactose (or the sugar under examination) and 5 ml. of water in an evaporating dish. Heat carefully on a water-bath until a vigorous reaction begins, marked by the evolution of red fumes of nitrogen peroxide. Remove the dish, and when the reaction has subsided, resume heating until most of the acid has been expelled and a syrup remains. Rinse the syrupy residue into a test tube by means of not more than 10 ml. of hot water. Allow to cool. A white crystalline precipitate of mucic acid separates out. Microscopically it appears as clusters of short, bright prisms, which dissolve readily in ammonium hydroxide.

(b) *Saccharic Acid*.—Remove the precipitated mucic acid (if any forms) by filtration, add about 3–5 gm. of anhydrous potassium

carbonate to the filtrate, and heat to ensure saturation. Pour the contents of the tube into a beaker, carefully acidify with 3–5 ml. of glacial acetic acid. On stirring and cooling the mixture, a white precipitate of potassium hydrogen saccharate separates out, and may be identified by its microscopic appearance of rosettes of sharp, brilliant needles.

If the first stage of the test yields no mucic acid it indicates that neither galactose nor lactose was present in the original material.

The Phenylhydrazine Test for Sugars.—Fill up about 1 cm. of a clean test tube with solid phenylhydrazine hydrochloride. Add twice as much solid sodium acetate. Then add 10 ml. of the sugar solution, and shake well. Heat the tube over a flame until the reagents are completely dissolved. Immerse the tube in boiling water, leave undisturbed for forty to sixty minutes, remove the flame, and let the tube cool slowly. If the reaction be positive the contents of the tube will have turned bright yellow, and a yellow crystalline sugar derivative, or osazone, will separate out as the tube cools.

Transfer some of the crystals by pipette to a slide, put on a cover slip, and examine microscopically.

Characteristics of the Commoner Osazones.—*Glucose*, *fructose*, and *mannose* yield the same osazone, **glucosazone**, which crystallises in yellow brushes or sheaves of slender needles; m.p. 205° C.

Galactosazone forms elongated strips and plates; m.p. 214° C.

Maltosazone occurs in stellate clusters of broad-bladed crystals; m.p. 205°–206° C. **Lactosazone** forms close tufts of short, fine crystals; m.p. 200° C. It is fairly soluble in hot water, and only separates out slowly on cooling. Osazone preparations from disaccharides are liable to be contaminated by monosaccharides liberated by hydrolysis during prolonged boiling. Among the pentoses, **arabinosazone** occurs in long curved threads and wisps; m.p. 167° C., while **xylosazone** occurs in long needles; m.p. 115°–158° C. Non-reducing sugars, such as sucrose and raffinose, do not yield osazones, owing to the absence of an aldehyde group. The osazones often can be identified by microscopic inspection, but it sometimes is necessary to find the melting point.

(a) Failure is often due to lack of sufficient phenylhydrazine; three molecules of which are required for each molecule of sugar (one for hydrazone formation, one for oxidation of an alcohol group in the hydrazone, and one for subsequent osazone formation).

(b) Owing to the instability of the free base, the hydrazine is used in the form of a hydrochloride. Since this is too acid, the mixture must be partly neutralised by addition of excess of sodium acetate.

(c) If no precipitate appears in the tube after 60 minutes' heating,

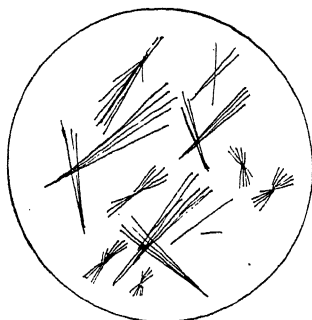
repeat the process for another 30 minutes, and let the tube cool slowly over-night. Glucose and fructose yield osazones much more rapidly than either maltose or lactose. If the mixture does not turn yellow after being heated, no osazone-forming carbohydrate is present.

(d) The advantage of the osazone test lies in its ability to reveal the presence of different sugars in a mixture, as well as to identify individual sugars.

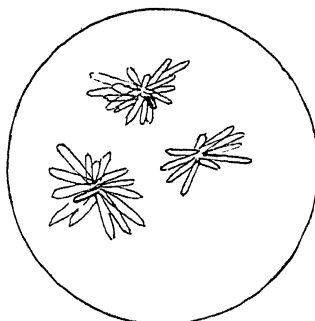
Summary of the Reactions of the Commoner Carbohydrates

Carbohydrate.	TEST.								
	Thymol.	Iodine.	Nitro-chromic.	Cu Reduction.	Osazone.	Ketose.	Yeast Fermentation.	Polarimetric Rotation.	HNO ₃ Oxidation.
	1	2	3	4	5	6	7	8	9
Pentose .	+	—	+	+	+	±	—	d or l	—
Hexose :									
glucose	+	—	+	+	G	—	+	d	Sc
fructose	+	—	+	+	G	+	+	l	—
galactose	+	—	+	+	Gl	—	+	d	Mc
Dihexoside									
maltose	+	—	+	+	M	—	+	d	Sc
sucrose	+	—	+	—	—	+	+	d	Sc
lactose	+	—	+	+	L	—	—	d	Mc + Sc
Hexosan :									
cellulose	+	±	—	—	—	—	—	(d)	—
starch	+	blue	—	—	—	—	—	d	—
e-dextrin	+	red	—	—	—	—	—	d	—
glycogen	+	red	—	—	—	—	—	d	—
inulin .	+	—	—	—	—	+	—	l	—

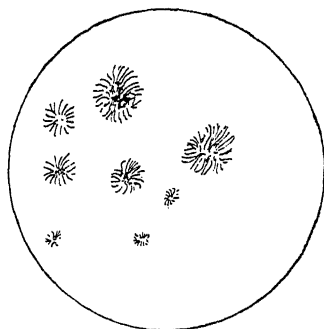
G = glucosazone; Gl = galactosazone; M = maltosazone; L = lactosazone.
 ± = reaction varies with conditions. Mc = mucic acid; Sc = saccharic acid.



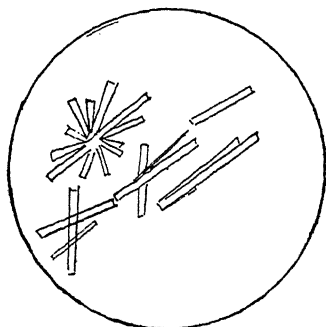
GLUCOSAZONE (X 50)



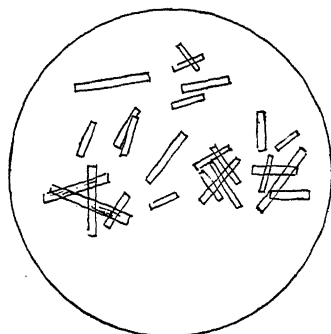
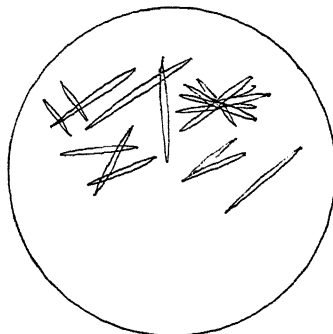
MALTOSAZONE (X 200)



LACTOSAZONE (X 200)



GALACTOSAZONE (X 200)

MUCIC ACID
(X 50)POTASSIUM HYDROGEN
SACCHARATE
(X 50)

Hydrolysis of Polysaccharides and Disaccharides

I. STARCH

Prepare a starch suspension by mixing 1 gm. of potato starch with 10 ml. of water. Starch is insoluble in cold water. Examine a drop of the suspension microscopically. Note characteristic laminated granules, which stain darkly with 0.1 per cent. iodine. Pour the starch suspension into about 90 ml. of boiling water. The granules disintegrate, forming a colloidal solution of starch.

(a) Hydrolysis of Starch by Strong Acids

(HCl at 100° C.)

Add 1 ml. of concentrated HCl to 25 ml. of 1 per cent. starch solution. Boil, and while boiling note the gradual loss of opalescence

as the polysaccharide is broken down. Prepare five test tubes, each containing 5 ml. of water and 1 drop of 1 per cent. iodine.

At intervals of five to ten minutes, transfer 1 ml. of the starch mixture to one of the test tubes. Compare the colour reaction with iodine as the hydrolysis proceeds. When all the polysaccharides are broken down an *achromic point* is reached, and no colour is given by iodine. Let the mixture cool. Add about 1 ml. of 20 per cent. sodium hydroxide to neutralise the excess of acid. Test the mixture for reducing sugars.

Course of Polysaccharides Hydrolysis

Carbohydrate	Starch → Dextrins → Maltose → Glucose
Iodine test	blue → red-brown → no change

(a) Do not mistake the yellow colour of the diluted iodine for a positive polysaccharide reaction.

(b) Addition of alkalis destroys the colour by combining with the iodine, acidification liberates iodine and restores the colour.

(c) Proteins interfere with the reaction by combining with the iodine, unless excess be added.

(d) Insoluble polysaccharides such as cotton-wool or starch granules do not react immediately with iodine. The colour given by dissolved cellulose depends on the nature of the solvent. In HCl and ZnCl₂ it gives an intense blue reaction.

(b) Hydrolysis of Starch by Enzymes

(Amylase at 40° C.)

Add 10 drops of an active pancreatic extract (which contains amylase) or malt extract (which contains diastase) to 10 ml. of 1 per cent. starch solution in a test tube. Make the mixture faintly alkaline to litmus paper or phenol red with a few drops of 1 per cent. sodium carbonate. Incubate the mixture in a water-bath at 40°. Prepare five tubes of dilute iodine solution, as before, but add 1 drop of 20 per cent. acetic acid in addition to each tube to keep the contents acid. Test the incubated solution at intervals of 10 minutes, and observe the colour reactions given with iodine.

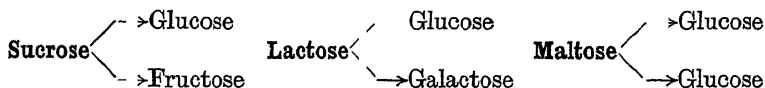
Notes.—(i) This method, conversely, is used to detect starch-splitting enzymes (amylases, diastases, ptyalin) in extracts and solutions.

(ii) The mixtures must not be boiled, or the enzyme will be destroyed.

(iii) To demonstrate the formation of sugar when starch is hydrolysed by enzymes, it is necessary to use pancreatic extract. Malt extract always contains reducing sugars, which obscures the test.

II. DISACCHARIDES

A disaccharide is hydrolysed by acids or enzymes into two monosaccharides.



(a) Hydrolysis of Sucrose by Acids

This has already been described as a test for sucrose (p. 111).

(b) Hydrolysis of Sucrose by Enzymes

(Sucrase or Invertase)

Incubate at 40°, 10 ml. of sucrose solution with 1 ml. of sugar-free invertase extract, prepared from yeast. After ten to fifteen minutes test samples of the mixture for reducing sugar, or demonstrate the presence of glucose and fructose by the osazone test, which, however, does not differentiate between these two sugars.

METHODS OF SUGAR ESTIMATION.

A. Copper Reduction.

For sugars in dilute (0.1–4.0 per cent.) solutions.

B. Yeast Fermentation.

For all common sugars, except lactose and pentoses.

A. Copper-reduction Methods.

I. Fehling's Method. (Blue → Red)

(1) Measure carefully by pipette 5 ml. of Fehling's reagent A into a 150 ml. conical flask. Add 5 ml. of reagent B, using a measuring cylinder to avoid contaminating the pipette. Add about 40–50 ml. of water. Set to boil over a small flame.

When several estimations are being done, it is convenient to mix a quantity of the reagents A and B in equal volumes beforehand. 10 ml. of the mixture are measured by pipette into the flask and diluted with 50 ml. of water.

(2) Fill a clean burette with the sugar solution. Run out sufficient to free the delivery tube of air bubbles. Note reading of burette.

(3) When the Fehling's reagent is boiling, run in the sugar solution, 0.5–1.0 ml. at a time, at short intervals, until the blue colour of the mixture changes to orange-brown owing to formation of cuprous hydroxide.

(4) Detection of the End-Point. The end-point is reached when all the blue colour has been discharged. This may be hard to detect in composite liquids, such as urine. Remove the flame,

and let the mixture cool. As the precipitate subsides, observe the clear solution.

Blue = underdone.

Colourless = done.

Yellow = overdone.

Resume titration.

Note reading of burette, and find volume of sugar added.

Note reading of burette. Start a fresh titration, and add less sugar solution.

Lane and Eynon Method for Determining the End-Point.—At the last stage of the reaction add a few drops of 0.1 per cent. methylene blue. This colours the mixture a dark blue, which persists until all the copper has been reduced. Then, the methylene blue in turn is reduced, and the colour of the solution clears rapidly. This change represents the end-point, the pigment being used as an internal indicator. It is important not to shake the flask during the latter stages of the reduction, as the leuco-methylene blue is easily re-oxidised by contact with air.

(5) *Calculation.*—10 ml. of mixed Fehling's reagent are completely reduced by 0.05 gm. of glucose. Hence, the volume of sugar solution used in the titration contains 0.05 gm. of glucose, or its equivalent.

Or, $x = \frac{5}{N}$, where x = percentage of **glucose** in the solution.
 N = number of ml. of solution required for complete reduction.

Notes.—(a) The estimation should be done at least twice, for accuracy.

(b) A small piece of "chalk" may be added if the boiling mixture begins to spurt.

(c) Mixed Fehling's reagent deteriorates on long keeping, and is stored separately as two solutions.

(6) *Calculation for Reducing Sugars other than Glucose.*—Should the reducing sugar be other than glucose an alteration must be made in the calculation formula, on account of the fact that the copper equivalent differs for different sugars.

(7) *Estimation of a Non-reducing Sugar such as Sucrose.*—The sucrose must first be hydrolysed into its constituents, glucose and fructose, the equimolecular mixture being termed "invert sugar." This may be done by adding sufficient H_2SO_4 to make approximately a 2 per cent. solution, and boiling for ten to twenty minutes. The mixture is then neutralised with NaOH , cooled, and made up to a known volume with water, and the sugar content estimated by the reduction method. The result is expressed either as percentage of invert sugar, x (invert sugar) $= \frac{5.1}{N}$, or as sucrose, knowing that 1.00 gm. invert sugar = 0.95 gm. sucrose.

To ensure that all the sucrose present has been "inverted," it is usual to repeat the hydrolysis for a longer interval of time, and see if the final results are the same.

Factor :	10 ml. of Fehling's reagent are reduced completely by :
x (glucose) $= \frac{5}{N}$	0.05 gm. glucose.
x (fructose) $= \frac{5.3}{N}$	0.053 gm. fructose.
x (lactose) $= \frac{6.76}{N}$	0.0676 gm. lactose.
x (maltose) $= \frac{7.4}{N}$	0.074 gm. maltose.

II. Benedict's Method. (Blue \rightarrow White)

(1) Measure carefully by pipette 25 ml. of Benedict's quantitative reagent into a 150 ml. flask. Add 3–5 gm. of anhydrous sodium carbonate (or as much as will fill 2 cm. of an ordinary test tube), and a small piece of "chalk" to keep the mixture from spurting.

(2) Boil the mixture over a moderate flame, and while boiling add the sugar solution from a burette, as in Fehling's method.

(3) The end-point is shown, as in the micro-method, by the replacement of all the blue colour by pure white. If it turns yellowish, too much sugar has been added.

Benedict's method has a sharper end-point and a greater specificity than Fehling's method. Its chief disadvantage is the liability of the mixture to spurt and boil over, unless the estimation be carried out rapidly, carefully and attentively.

(4) *Calculation.*—Benedict's reagent is of such a strength that 25 ml. are reduced by 0.05 gm. glucose (or, 25 ml. Benedict's reagent = 10 ml. Fehling's reagent). As before, $x = \frac{5}{N}$.

Where N = number of ml. of sugar solution added ;

x = percentage of glucose.

For the other reducing sugars the appropriate equation must be used.

(a) It is necessary to keep Benedict's solution boiling during the estimation, adding water if the mixture becomes very concentrated, and starts to spurt.

(b) The use of methylene blue to sharpen the end-point is inadvisable. The Benedict reaction is marked by a colour change and not by the subsidence of a precipitate.

(c) In titrating sugar solutions weaker than 0.3 per cent., use 10 ml. reagent and 10 gm. anhydrous carbonate.

Here, x (glucose) $= \frac{5}{2.5N} = \frac{2}{N}$.

III. The Drop Method

To avoid the difficulties, due to super-heating and spurting, which unpractised workers encounter when using Benedict's method, the following modification has been made. It is rapid, and can be carried out by means of a calibrated pipette instead of a burette.

(1) Fill up about 1 cm. of a clean test tube with anhydrous sodium carbonate. Add carefully, by pipette, 5 ml. of Benedict's *quantitative* reagent.

(2) Boil over a small flame until the carbonate is dissolved.

(3) Add the sugar solution from a burette or a pipette fitted with a rubber bulb, one drop at a time. Boil for a few seconds after addition of each drop. Count the drops.

(4) When the blue colour has almost gone, proceed slowly, and let the tube cool in the rack for about one minute before addition of the next drop.

(5) The end-point is obtained when the mixture is pure white. If excess of sugar has been added, the mixture becomes yellow. The average time of an estimation should not exceed three to four minutes. The estimation should be repeated.

(6) *Calculation.*—5 ml. of Benedict's quantitative reagent are reduced by 0.01 gm. of glucose. Hence, the solution added contains 0.01 gm. of glucose, or its equivalent.

$$x \text{ (glucose)} = \frac{d}{n};$$

where x = percentage of glucose in the solution.
 n = number of drops required for complete reduction.
 d = number of drops in 1 ml., usually 20.

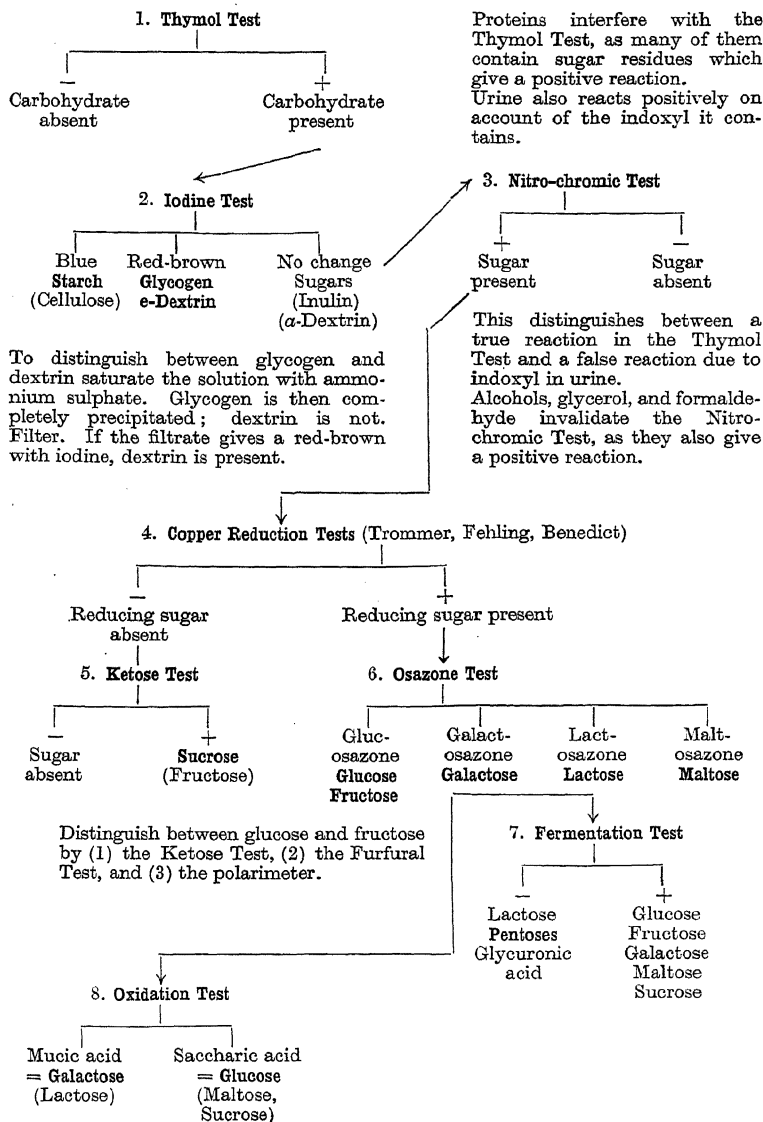
$$x \text{ (lactose)} \quad 1.35d \quad \quad \quad 1.48d$$

" " " "

Note.—The value for d can be found experimentally by counting the number of drops delivered from a known volume of the solution in the pipette or burette. It ranges from 18–21 per ml., and 20 is a usual average value for a burette.

Estimation of Sugar in Concentrated Solution.—If the solution contain more than 5 per cent. of reducing sugar (*i.e.*, if less than 1 ml. reduces all the copper reagent), it must be diluted 1 : 5 or 1 : 10 to yield an accurate result with either Fehling's or Benedict's method. For dilution 1 : 10, measure 10 ml. of sugar solution exactly by pipette into a 100 ml. measuring flask. Fill up carefully to the 100 mark. Mix thoroughly. Rinse out the burette twice with the diluted sugar solution. Fill the burette, and estimate as before. The final result must be multiplied by 10 to obtain the percentage of sugar in the original undiluted solution.

Method for Identifying One of the Common Carbohydrates



GENERAL REFERENCES

- ARMSTRONG, E. F., and H. E. ARMSTRONG (1931), "Glycosides." London.
- ARMSTRONG, E. F., and H. E. ARMSTRONG (1934), "Carbohydrates." London.
- BARTON-WRIGHT, E. (1933), "Recent Advances in Plant Physiology." London.
- FEARON, W. R., and D. MITCHELL (1932), "Nitro-chromic acid reaction." *Analyst*, 57, 372.
- HAWORTH, W. N. (1929), "Constitution of the Sugars." London.
- HEUSER, E. (1938). "Cellulose." *Organic Chemistry*, Ed. Gilman, 2, 1534.
- IRVINE, J. C. (1927), "Structural study of carbohydrates." *Chem. Rev.*, 4, 203.
- LEVENE, P. A. (1925), "Hexosamines and Mucoprotamines." London.
- NORMAN, A. G. (1937), "Biochemistry of Cellulose." London.
- PRINGSHEIM, H. (1932), "Chemistry of the Monosaccharides and the Polysaccharides." New York.
- PRYDE, J. (1931), "Recent Advances in Biochemistry." London.
- RAYMOND, A. L. (1938), "Carbohydrates." *Organic Chemistry*, Ed. Gilman, 2, 1477.
- WHITTIER, E. O. (1925), "Lactose." *Chem. Rev.*, 2, 85.
- WOLFROM, M. L. (1938), "Carbohydrates." *Organic Chemistry*, Ed. Gilman, 2, 1399.

CHAPTER VIII

PROTEINS

Definition.—Proteins are complex natural compounds formed by the condensation of mixed amino acids. They are present in all plant and animal tissues, and represent the stable organic form assumed by nitrogen during its association with the living organism.

Class Characteristics.—(1) All proteins contain C, H, O, and N, usually in the following amounts, expressed as percentage :—

C, 50–55 ; O, 21–23·5 ; N, 15–17·6 ; H, 6·5–7·0.

The high content of nitrogen is a feature of the protein family of biological compounds.

Many proteins also contain S and P, depending on the particular amino acid units or esters present.

(2) All proteins are of high molecular weight and colloidal dimensions.

(3) All proteins give a rose-violet colour on addition of dilute copper sulphate and sodium hydroxide. This is the copper protein or “biuret” test.

(4) All proteins on hydrolysis yield mixtures of α -amino acids, the type formula of which is $R\cdot CH(NH_2)\cdot COOH$.

(5) With the exception of the group of pigmented chromoproteins, the proteins as a family have no characteristic colour, odour, or taste.

Proteins are classified *biochemically*, according to solubility, coagulability, and amino acid content ; and *biologically*, according to natural distribution.

BIOCHEMICAL CLASSIFICATION OF PROTEINS

A	B	C
Simple Proteins	Conjugated Proteins	Derived Proteins
(1) Protamines.	(8) Phosphoproteins	(15) Denatured Proteins
(2) Histones	(9) Nucleoproteins	(16) Cleavage Products :
(3) Gliadins	(10) Glycoproteins	(a) Metaproteins
(4) Glutelins	(11) Chromoproteins	(b) Proteoses
(5) Sclerins	(12) Zymoproteins	(c) Peptones
(6) Albumins	(13) Hormoproteins	(d) Peptides
(7) Globulins	(14) Virus proteins	(e) Amino acids, etc.

CLASS PROPERTIES OF THE PROTEINS

A. SIMPLE PROTEINS

(1) **Protamines.**—The simplest natural proteins, found only in fish sperm. At least twelve protamines are known, each being made up of four to ten different species of amino acid, one of which is always arginine. Thus *salmin* (from salmon sperm) has a minimal molecular weight of 2,855, and on hydrolysis liberates 14 molecules of arginine, 3 of proline, 3 of serine, and 1 of valine. All protamines are water soluble and strongly basic, owing to their high content of diamino acids.

(2) **Histones.**—Basic proteins resembling protamines, but containing a greater variety of amino acid units. They only occur in animal tissues, usually in union with nucleic acid.

Examples are :—

- (a) *Thymus histone*, a powerful anti-coagulant of blood, originally isolated from the thymus gland of the calf.
- (b) *Nucleohistones* from cell nuclei of various tissues, including sperm cells.
- (c) *Globin*, the protein component of the blood pigment, hæmoglobin.

(3) **Gliadins or Prolamins.**—These are peculiar to plants, and occur largely in the seeds of cereals. They are insoluble in water and in 90 per cent. alcohol, but have the special property of being soluble in 70–80 per cent. aqueous alcohol. They are not coagulated by heat. Important prolamins are : hordein, from barley ; zein, from maize ; and gliadin, from wheat and rye.

As a class, gliadins are characterised by their high content of proline, which accounts for the original name prolamine.

(4) **Glutelins.**—Vegetable proteins found chiefly in cereal grain. They are insoluble in water and aqueous alcohol in all concentrations, but may be dissolved by dilute acids or alkalis. They are not coagulated by heat. *Glutenin*, from wheat, and *oryzenin*, from barley, are the most definite members of the class.

When wheat flour is made into a paste with water the gliadin present forms an adhesive and binds together the glutenin. The mixture is termed *gluten*, and is responsible for the production of dough. Gluten makes up about 6–12 per cent. of wheat flour.

(5) **Sclerins, Scleroproteins, or Albuminoids.**—Insoluble proteins found in the skeletal, connective, and epidermal tissues of animals. They do not occur in plants. Scleroproteins are very stable, and resist acid and alkaline hydrolysis, but ultimately are resolved into amino acids.

- (i.) *Collagens* or *gelatin precursors*, found in bone, cartilage, and the "white fibres" of connective tissue. Collagens are hydrolysed by boiling with concentrated acids or alkalis, or by superheated steam. They are attacked by the gastric enzyme pepsin, but not by trypsin. *Gelatin* is the characteristic product of intermediate hydrolysis. Collagens are rich in the simple amino acid glycine, the yield of which may be as high as 25 per cent. They lack the biologically important amino acids, cystine, tyrosine, and tryptophane.
- (ii.) *Elastins*, found in the "yellow" or elastic fibres of connective tissue, in cartilage and ligaments. They differ from collagens in being readily attacked by both pepsin and trypsin. The end-products of hydrolysis are especially rich in glycine (25 per cent.) and leucine (20 per cent.).
- (iii.) *Keratins*.—These scleroproteins are characteristic of epidermal tissue, and occur in hair, wool, feathers, claws, horns, and nails. They are the most stable of all proteins, and are well adapted to withstand the environment. Insoluble in water, dilute acids and alkalis, they resist most of the proteoclastic enzymes, including pepsin and trypsin.

Keratins are dissolved by concentrated alkalis, and by metallic sulphides, a property that underlies the use of barium or calcium sulphide as a depilatory. Chemically, true keratins are rich in the amino acids histidine, lysine, and arginine, which are present in the ratio 1 : 4 : 12. The tyrosine and cystine contents also are usually high; tyrosine being about 3–4 per cent., and cystine reaching a maximal value of 8–14 per cent. in wool.

(6) *Albumins*.—The group of water-soluble, **heat-coagulable** proteins found in animals and plants. Important members are *ovalbumin*, from egg-white; *serum albumin*, from blood; *lactalbumin*, from milk; *myoalbumin*, from muscle; *leucosin*, from wheat, rye, and barley; *legumelin*, from pea, bean, and lentil.

As a class they undergo a characteristic irreversible coagulation when heated to about 75° C. in solution. Coagulation is most marked in slightly acid solutions (pH6–pH5), and is retarded by alkalis. It forms an important test for higher proteins.

- (i.) *Ovalbumin* makes up the greater part (10–13 per cent.) of egg-white. It can be obtained in crystalline form by half-saturation with ammonium sulphate at pH 4.58. It is lævo-rotatory in solution, $[\alpha] = -35.5^\circ$.
- (ii.) *Serum albumin* occurs in serum (4–6 per cent.), lymph, and other tissue fluids. Like ovalbumin it coagulates about 75° C., but differs in being more lævo-rotatory, $[\alpha] = -56^\circ$.

The vegetable albumins resemble serum albumin in most properties, but differ in that they contain glycine, an amino acid that is often absent from animal albumins, other than lactalbumin.

(7) **Globulins.**—Heat-coagulable proteins, insoluble in pure water but soluble in presence of electrolytes. They accompany albumins in many tissues, and carry corresponding names : *ovoglobulin*, *serum globulin*, *lactoglobulin* and *myoglobulin*. *Fibrinogen*, from blood plasma, is a globulin, and the majority of vegetable proteins and seed proteins are of the globulin type. Examples are : *legumin*, from seeds of pea, bean, and lentil ; *excelsin*, from Brazil nut ; *edestin*, from hemp seed. Reserve protein of seeds is usually a globulin, many can be crystallised.

Distinction between Globulins and Albumins.—Globulins, unlike albumins, are insoluble in pure water, and must be extracted from tissues by means of dilute (5 per cent.) salines, such as NaCl, MgSO_4 , etc. Removal of the salt by dialysis precipitates the globulin. All animal globulins precipitate on full saturation with MgSO_4 , or on half-saturation with $(\text{NH}_4)_2\text{SO}_4$, vegetable globulins, however, are not completely precipitated by these reagents. Albumin and globulin usually occur together in animal tissues and fluids, such as blood serum. Separation may be effected by :—

- (1) Addition of excess of water, which precipitates the less soluble globulin.
- (2) Dialysis, which precipitates the globulin by removing the salts that keep it in solution.
- (3) Addition of an equal volume of saturated (*i.e.*, 80 per cent.) ammonium sulphate ; this half-saturates the protein solution, and precipitates the globulin.

None of these methods can be considered as exact, the sharpness of the separation depending on many factors, such as the pH of the mixture and the relative concentrations of proteins present. The individuality of the animal globulins is not yet clearly established, serological tests indicate that serum globulin and lactoglobulin are identical in the same species of animal.

The Albumin-Globulin Complex.—Albumins and globulins are distinguished from all the other proteins by being coagulated by heat ; apart from this, their individual characteristics depend largely on the method employed to separate them from one another, and it is believed that they occur naturally in the form of an unstable association, or protein complex, making up the colloidal matrix of cells and tissues, including the blood plasma. The term *orosin* is sometimes used to denote this heat-coagulable protein complex.

B. CONJUGATED OR COMPOUND PROTEINS

These consist of proteins united to a non-protein, or *prosthetic*, group.

(8) **Phosphoproteins.**—Compounds of protein and phosphoric acid. The phosphoric radicle is united in two ways: (i) as a peptide linkage, —NH—OP— , attacked by trypsin; and (ii.) as an ester linkage, —CO—OP— , attacked by phosphatase. Phosphoproteins are found in milk and in egg-yolk, and are of primary importance in the nutrition of the young animal. They are characterised by the presence of all the essential amino acids, and by their high content of glutamic acid, which may reach 20 per cent.

The chief phosphoproteins are :—

- (a) **Caseinogen**, a characteristic constituent of all milk, the value ranging from about 1 per cent. in human milk up to nearly 9 per cent. in the milk of the cat. Caseinogen is insoluble in water, but readily dissolves in dilute alkalis to form soluble caseinates; calcium caseinate being the form present in milk. Caseinates may be precipitated unchanged by addition of excess of alcohol, or they may be decomposed by addition of dilute acid, whereupon the liberated caseinogen is precipitated.

This occurs when milk becomes acid, or “sour,” owing to bacterial decomposition of lactose to lactic acid.

Caseinogen is precipitated completely about pH4.6, if more acid be added the protein redissolves.

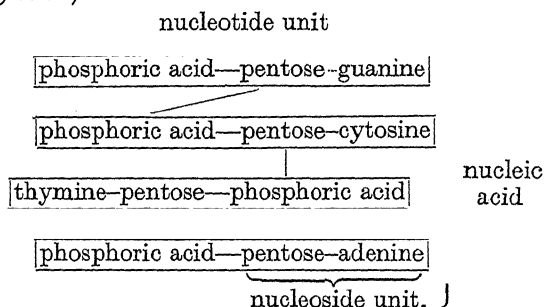
Caseinogen solutions are not heat-coagulable, but on the addition of the enzyme *rennin*, the protein is changed into another form, casein, or paracasein, which yields an insoluble coagulum with calcium salts. This coagulation of milk occurs in the stomach as a preliminary stage in the digestion. It is distinguished from the clotting of sour milk by being (i.) irreversible, and (ii.) due to the action of a special enzyme, the chymase, or rennin, in gastric juice.

- (b) **Vitellin**, the chief protein of avian egg yolk, is a phosphoglobulin, being heat-coagulable, and insoluble in water but soluble in dilute salines.
- (c) **Livetin**, another phosphoglobulin, makes up 20–25 per cent. of the total yolk protein (or 0.6–0.9 gm. livetin) in the egg of the domestic fowl. It contains 0.05 per cent. P, which corresponds to a molecular weight of 64,000 for one atom of P per molecule.

(9) **Nucleoproteins.**—The nuclei of cells are characterised by the presence of strands of a material staining deeply with basic dyes,

and for this reason termed *chromatin*. Chromatin is the carrier of the genes, or inheritance factors, and is composed chiefly of nucleoprotein, a compound of histone or protamine and nucleic acid. The protein component differs not only in the different species of animals, but even in the different tissues of the same species, and is a determinant of biological individuality.

Nucleoproteins are almost insoluble in water, but dissolve in alkalis, and by this means can be extracted from tissues rich in nuclei, such as thymus and pancreas. They were obtained originally from pus, which is an emulsion of white corpuscles. Another rich source is the sperm of fishes. On acid or enzyme hydrolysis, nucleoproteins are resolved into protein and nucleic acid, which further hydrolysis converts into four units, or **nucleotides**. Each nucleotide is the phosphoric ester of a **nucleoside**, or pentose derivative of an amino purine (*adenine* or *guanine*), or an amino pyrimidine (*cytosine* or *methyl cytosine*).



Thus, a nucleic acid is a mixed tetranucleotide, and is derived from: two purines, two pyrimidines, four molecules of a pentose and four molecules of phosphoric acid.

The pentose is either D-ribose, $C_5H_{10}O_5$, or D-2-desoxyribose, $C_5H_{10}O_4$, and accordingly two types of nucleic acid are known.

1. *Thymo-nucleic acid*, found in thymus, spleen, pancreas, liver, kidney, fish sperm, and other sources of animal chromatin. In this form of the acid the pyrimidines are cytosine and thymine and the sugar is desoxyribose, which gives a positive Feulgen colour test (p. 349).

2. *Yeast nucleic acid*, found in yeast, wheat germ and other sources of plant chromatin, yields the pyrimidines cytosine and uracil, and the sugar D-ribose, which does not give Feulgen's test.

It was formerly thought that yeast nucleic acid was the typical plant nucleic acid and thymo-nucleic acid was only found in animals, but this distinction has had to be abandoned on account of the discovery of the ribose nucleic acid in many animal tissues.

(10) **Glycoproteins or Mucoproteins**.—Compounds of protein and neutral mucopolysaccharides. The glycoproteins were formerly classified as mucins and mucoids, but the term "mucin" is

ambiguous and applies to many viscous secretions, some of which owe their properties to free mucopolysaccharides. Glycoproteins are represented by : ovomucoids from egg white, serum mucoid, thyroglobulin, and the gonadotrophic hormone found in the urine of pregnancy.

Glycoproteins dissolve in water to form very viscid solutions not coagulated by heat. They resist the attack of ordinary digestive enzymes, but are hydrolysed by hot alkalis, with liberation of hexosamine from the mucopolysaccharide residue (p. 97).

(11) **Chromoproteins.**—Compounds of protein and a pigment, usually a metallic derivative of porphyrin.

(i) *Hæmochromes*, or blood pigments, the chief of which is **hæmoglobin**, the red chromoprotein found in the blood cells of all vertebrates. It is composed of 94 per cent. of a histone, *globin*, united to the ferrous porphyrin, *hæm*. Chlorocruorin is a green chromoprotein found in some marine worms. Hæmocyanin occurs as a blue hæmochrome in crustaceans.

(ii.) *Cytochromes*, hæm complexes found in all aerobic cells, and forming part of the cell oxidation apparatus.

(iii.) *Phytochromes*: plant chromoproteins, including phycoerythrin, a red pigment, and phycocyan, a blue pigment, both found in seaweeds.

(iv.) *Flavoproteins*.—The “yellow” respiratory catalyst, discovered by Warburg, is a chromoprotein of D-riboflavin (vitamin B₂).

(12) **Zymoproteins.**—Heat-labile proteins having catalytic properties. This group includes the important enzymes: pepsin, papain, chymase, catalase, peroxidase, and urease.

(13) **Hormoproteins.**—Several of the internal secretions of the human body have been found to be proteins or protein derivatives. Chief among these protein hormones are : insulin, secretin, thyroglobulin, prolactin and other pituitary factors, parathyrin. The simple autacoids, adrenaline and thyroxine are amino acid derivatives.

(14) **Virus Proteins.**—A virus may be defined as an organism that through parasitism has lost structure and substance, and can only function as a living unit when in contact with the cytoplasm of living tissue. Specific diseases of animals or plants are now ascribed to infection by filterable viruses, several of which have been obtained in crystalline form by Stanley and others, and shown to be complex nucleoproteins. The diameter of these viruses, as calculated from ultra-filtration data, ranges from 10 m μ (foot-and-

mouth disease), or 15 m μ (tobacco mosaic) up to 175 m μ (vaccinia), the maximum molecular weight being $2,300 \times 10^6$ (vaccinia).

C. DERIVED PROTEINS

Products obtained by the denaturation and cleavage of natural proteins. They represent stages in protein digestion and synthesis.

(15) **Denatured Proteins.**—Denaturation is marked by complete loss of solubility in water and in neutral salt solutions. It is usually irreversible, and is brought about by many agents, including heat, strong acids, and the prolonged action of alcohol. Denaturation may be effected also by specific catalysts, as in coagulation of caseinogen by rennin, or the conversion of fibrinogen into insoluble fibrin during the clotting of blood.

Heat-Coagulation.—Albumins and globulins differ from all the other proteins in being coagulated when heated in aqueous solution. The change is preceded by heat-denaturation, which is favoured by an acid reaction; then the insoluble protein particles flocculate or coagulate, a change which takes place best in presence of neutral electrolytes, such as NaCl, about pH 5·7–pH 5·9.

Heat-coagulation will not take place in an alkaline solution, or in a strongly acid solution deficient in salts. Hence, in testing for albumin or globulin the solution is first slightly acidified with dilute acetic acid, and treated with sodium chloride. The coagulation temperature depends on the nature of the protein, the reaction of the mixture, and the electrolytes present.

Blood serum at pH 5·7 coagulates about 75° C.; egg-white coagulates about 62° C. Heat-coagulation is an important group test, and a means of detecting traces of higher protein in urine.

(16) **Protein Cleavage Products.**—In denaturation and coagulation the protein molecule is rearranged but not decomposed; in the subsequent changes of cleavage, the molecule is fragmented in stages until the final end-products are reached. Four intermediate and somewhat artificial levels of hydrolysis are recognised:—

(a) *Metaproteins.*—Cleavage products not coagulable by heat, insoluble in water at pH 6·0, but soluble in greater concentrations of acid or alkali. When dissolved in either of these reagents they are termed acid metaprotein and alkali metaprotein, respectively. They resemble higher proteins in giving a violet colour reaction with copper sulphate and sodium hydroxide. Metaproteins from albumin and globulin give a positive Rothera test (p. 150), owing to the unmasking of —SH groups during early hydrolysis.

(b) *Proteoses.*—Cleavage products not coagulable by heat, soluble

in water, and not precipitated at pH 6.0, but completely precipitated by saturation with sodium sulphate at 33° C. Unlike metaproteins and higher proteins, the proteoses give a rose colour reaction in the copper protein test.

- (c) *Peptones*.—Cleavage products resembling proteoses, but not precipitated by saturation with sodium sulphate. They give a rose colour reaction in the copper protein test, and are completely precipitated by strong tannic acid. Peptones resist the action of the proteoclastic enzymes, pepsin and pure trypsin, but are attacked by the peptidases, such as erepsin, found in the small intestine.
- (d) *Peptides*.—Simple hydrolytic products, mostly soluble in water and not precipitated by tannic acid. They give a rose colour in the copper protein test, and are precipitated by excess of alcohol.

Proteoses, peptones and peptides constitute the sub-group of **lower proteins**, as distinct from their precursors, the higher proteins.

- (e) **Amino Acids**.—The chief end-products of protein hydrolysis. The type formula is $R \cdot CH(NH_2) \cdot COOH$, and they are derived from simple aliphatic acids by replacement of H in the α -position by the *amino group*, NH_2 . Many amino acids carry other groups attached to the terminal C atom on the left end of the aliphatic chain. At least thirty different amino acids have been recovered from the products of protein hydrolysis.
- (f) **Imino Acids**.—Two imino acids, *proline* and *hydroxyproline*, have been isolated.
- (g) **Hexosamines**.—*Chondrosamine* and *chitosamine* occur among the products of glycoprotein hydrolysis (cf. p. 99).
- (h) **Ammonia** is an invariable product of alkaline hydrolysis, and is believed to come from the decomposition of structural units containing the amide group, $-CO \cdot NH_2$.

Protein hydrolysis may be brought about by :—

- (i.) Boiling with strong acids, such as 5–25 per cent. HCl. This is the principal general method in use, although it involves loss of at least two important amino acids, namely, tryptophane and cystine.
- (ii.) Boiling with alkalis. This causes loss of at least three acids, citrulline, arginine, cystine, and, possibly, histidine.
- (iii.) Zymolysis or hydrolysis by enzymes. This is highly efficient within the organism, but slow and incomplete in the laboratory. It is mostly used in the preparation of the less stable amino acids, such as tryptophane.

The Carbohydrate Content of Proteins.—Egg albumin, serum albumin and many other proteins give a purple colour on being warmed with thymol and hydrochloric acid, showing the presence of a carbohydrate. This reaction persists even when the protein has previously been purified by repeated crystallisations, and has been traced to the constant presence of a sugar residue, mannose or galactose, in the protein molecule.

Hexose Content of Proteins

Egg-white protein :

ovoglobulin	.	4.0 per cent. mannose.
ovalbumin	.	1.7 per cent. mannose.
ovomucin	.	14.9 per cent. mannose + galactose.

Serum protein :

seralbumin	.	0.47 per cent. mannose + galactose.
serglobulin	.	1.82 per cent. mannose + galactose.

Milk proteins :

casein	.	0.31 per cent. galactose.
lactalbumin	.	0.44 per cent. galactose.

CHAPTER IX

AMINO ACIDS AND PROTEIN STRUCTURE

WHILE the number of natural proteins is not yet known, and must be as great, if not greater, than the total number of plant and animal species, since species individuality seems to reside in protein structure, the number of different amino acids isolated from proteins is about thirty, and of these twenty-five are generally accepted as protein structure units. Variety in protein architecture is obtained by variety in the order of arrangement of these units within the protein molecule.

Definition.—An amino acid is an organic acid in which one or more residual hydrogen atoms have been replaced by amino groups. Chemically, the majority of biological acids are α -amino acids, the $-\text{NH}_2$ group being attached to the C atom next the terminal carboxyl group.

Type formula : $R \cdot \text{CH} \cdot \text{COOH}$

where R , the hydrocarbon residue, may represent aliphatic, aromatic or heterocyclic radicles.

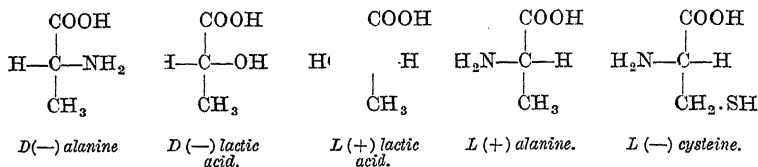
General Properties of the Amino Acids.—All the natural acids are colourless crystalline solids. All, except cystine, leucine, and tyrosine, are readily soluble in water ; and all, except proline and hydroxyproline, are sparingly soluble in alcohol. All are optically active, except glycine, the α -carbon of which is not asymmetric. The dextro acids are : alanine, valine, iso-leucine, glutamic acid, hydroxyglutamic acid, aspartic acid, arginine, citrulline, and lysine. The others are lævo-rotatory.

While amino acids may be classified as dextro (+) or lævo (−) rotatory, a more logical plan has been worked out by Levene, who refers them to a parent molecule, tartaric acid, which exists in two optically isomeric forms, namely D-tartaric acid and L-tartaric acid, so called because they are spatially related to D-glucose and L-glucose, respectively. Actually D-tartaric acid is the lævo-rotatory isomer, and to avoid confusion of notation, the two acids are written D (−) tartaric acid and L (+) tartaric acid.

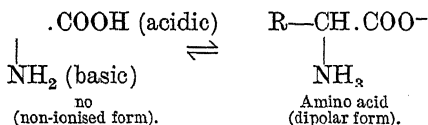
From L (+) tartaric acid is derived L (+) lactic acid, which is

also obtainable from the natural amino acid (+) alanine. Hence it is concluded that alanine is an L-acid, and a stereochemical descendant of L-glucose.

Almost all natural amino acids are of the L-series, a very notable exception being D-glutamic acid, found in malignant tissue protein (Kögl and Erxleben, 1939).



Dipolar Form of the Amino Acids.—The monoamino-monocarboxy acids are neutral in solution, and are very weak electrolytes. At the same time they are able to neutralise either acids or bases. This property, termed *amphotericity*, is due to the presence of an acid and a basic group in the same molecule. In aqueous solutions amino acids ionise to form a dipolar or **zwitter-ion**, having two equal charges of opposite electric sign, and tending to migrate neither to anode nor cathode when a current is passed through the solution.

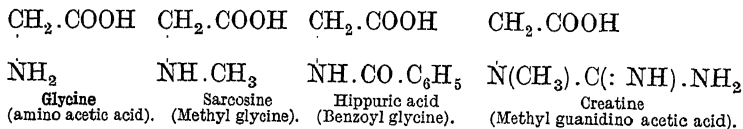


Acids are neutralised by the —COO^- group of the amino acid, which can combine with H^+ to form —COOH . Alkalies are neutralised by the —NH_3^+ group, which can donate H^+ by reverting to —NH_2 .

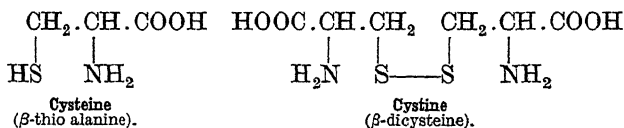
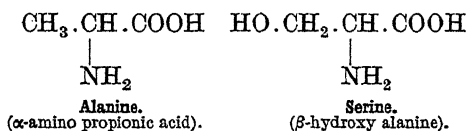
CLASSIFICATION OF THE AMINO ACIDS

The simplest classification refers each acid to the parent aliphatic acid.

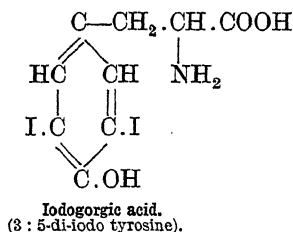
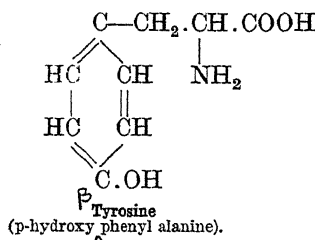
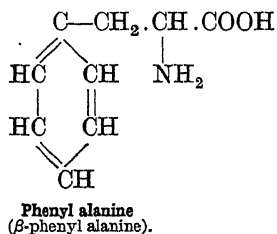
(1) Acids derived from Acetic Acid : $\text{CH}_3\cdot\text{COOH}$.



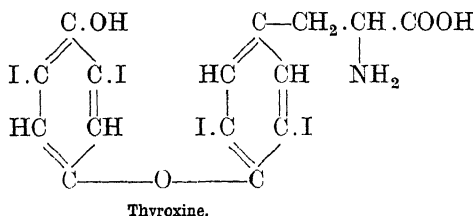
Glycine is the only one of these derivatives found as a protein unit. **Sarcosine** is a hydrolysis product of creatine. **Hippuric acid** and **creatine** are amino acid derivatives.

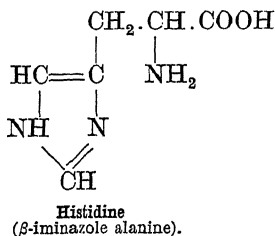
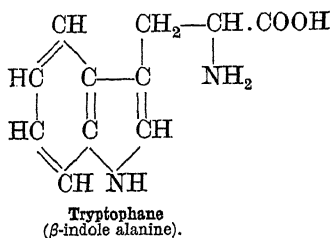
(2) Amino Acids derived from Propionic Acid : $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{COOH}$.


Cyclic Amino Acids derived from Alanine.—These may be regarded also as amino acid derivatives of aromatic and other nuclei.

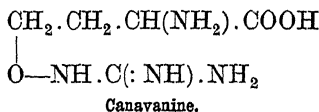
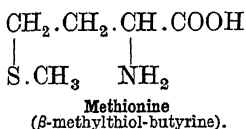
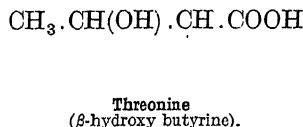
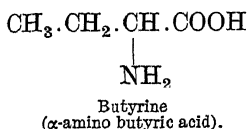


Iodogorgic acid has only been found in the scleroprotein of sponges. It is of special interest on account of its chemical relationship to thyroxine, the characteristic amino acid of the thyroid gland.





(3) **Amino Acids derived from Butyric Acid : $\text{CH}_3\text{.CH}_2\text{.CH}_2\text{.COOH}$**

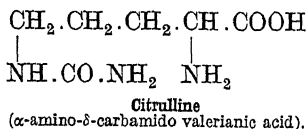
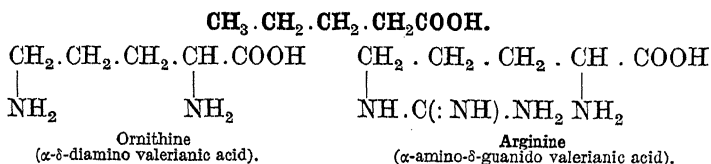


Threonine was isolated by Rose, in 1935, from casein.

Methionine, like cystine, is a sulphur-containing amino acid, and the source of this element in animal nutrition.

Canavanine, isolated from soy bean meal, resembles arginine in being a guanidine derivative and yielding urea on alkaline hydrolysis

(4) **Amino Acids derived from Valerianic Acid :**



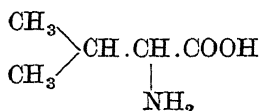
Arginine occurs in many proteins, and may be an essential part of the molecular pattern of all proteins ; on hydrolysis it yields ornithine and urea.

Citrulline occurs free in the melon (*Citrullus*) ; and also as a protein constituent.

(5) Amino Acid derived from iso-Valerianic Acid.

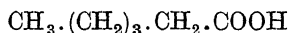


Iso-valerianic
acid.

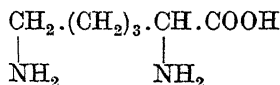


Valine
(α -amino iso-valerianic acid).

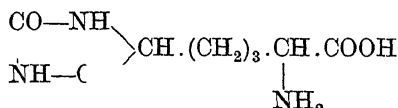
(6) Amino Acids derived from Caproic Acids.



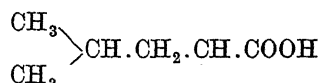
Caproic acid.



Lysine
(α - ϵ -diamino caproic acid)



Prollysine.

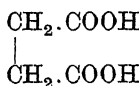


Leucine
(α -amino iso-caproic acid).

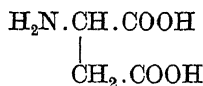


Iso-leucine
(β -methyl-valine).

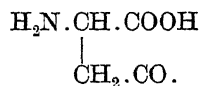
(7) Amino Acids and Amides derived from Succinic Acid.



Succinic acid.

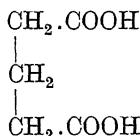


Aspartic acid.
(amino succinic acid).

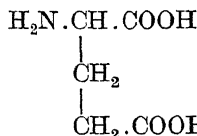


Asparagine
(β -amino succinamide).

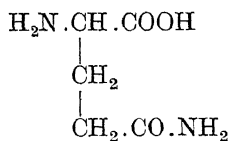
(8) Amino Acids and Amides derived from Glutaric Acid.



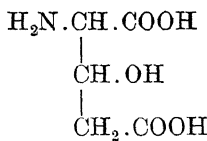
Glutaric
acid.



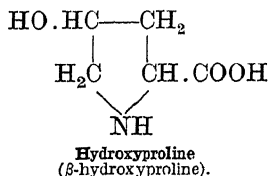
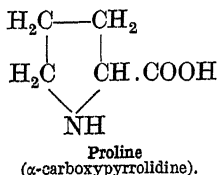
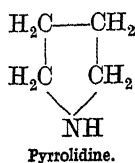
Glutamic acid
(α -amino glutaric acid).



Glutamine
(β -amino glutamide).



β -hydroxy glutamic
acid.

(9) **Imino Acids derived from Pyrrolidine.**

According to Wada (1933), *citrulline* is a precursor of proline, and *prolysine* is a precursor of lysine in protein hydrolysis.

Physiological Classification of Amino Acids.

(1) Essential for life of higher animals : *threonine, leucine, iso-leucine, phenylalanine, tyrosine, histidine, lysine, valine, methionine, tryptophane.*

(2) Non-essential. The remaining amino-acids, with the possible exception of *arginine*, or some unrecognised source of the guanidine nucleus.

(3) *Glucogenic* amino acids, capable of giving rise to glucose in the animal body.

(4) *Ketogenic* amino acids, giving rise to aceto-acetic acid when perfused through a surviving liver.

Summary of the Amino Acids

In the dicarboxyl acids the presence of a second carboxyl group dominates the single amino group, and for this reason aspartic, glutamic, and hydroxyglutamic acids have an acidic reaction in aqueous solution. The diamino acids, with the exception of *citrulline*, are strongly basic, owing to the presence of two amino groups and only one carboxyl.

Fractionation of the Products of Protein Hydrolysis.—The amino acids can be separated analytically into three groups :—

(1) *The basic group*, precipitated by phosphotungstic acid. This group includes the three diamino acids : *arginine, histidine, lysine*, along with *cystine* and the imino acids, *proline* and *hydroxyproline*.

(2) *The acidic group*, precipitated as salts after addition of calcium hydroxide and alcohol up to 80 per cent. The group consists of the three dicarboxy acids, *aspartic, glutamic, and hydroxyglutamic*.

(3) *The neutral group* of monocarboxy-monoamino acids. No complete qualitative method of separation is known. On concentration, *tyrosine, leucine, and cystine*, the least soluble of the acids, crystallise out.

Cystine crystallises in characteristic hexagonal plates that are seen in urine and in urinary calculi in the rare condition of *cystinuria*. On reduction *cystine* is converted into two molecules of *cysteine*, which is readily soluble in water.

Essential in diet.	Glucogenic.	Ketogenic.	Amino Acid.	Character.			
—	()	()	Citrulline.	Basic amino acids. 1 carboxyl and more than 1 basic group.			
()	()	()	Canavanine.				
+?	+	—	Arginine.				
+	—	+	Histidine.				
+	—	—	Lysine.				
?	?	?	Thyroxine.	Contain iodine.	Contain benzene ring.	Aromatic amino acids.	Neutral amino acids. 1 carboxyl and 1 amino group.
?	?	?	Iodogorgic acid.				
?	—	+	Tyrosine.	Phenolic.			
+	—	+	Phenylalanine.				
+	—	—	Tryptophane.	Indolic.			
()	+	—	Proline.	Imino acids.			
?	+	—	Hydroxy-proline.				
—	+	—	Cystine.	Contain sulphur.	Aliphatic acids.		
+	()	()	Methionine.				
+	—	+	Leucine.				
+	—	—	Iso-leucine.				
+	—	—	Valine.				
+	()	()	Threonine.	Hydroxy acids.			
()	+	—	Serine.				
()	+	—	Alanine.				
—	+	—	Glycine.				
—	+	—	Glutamic acid.	Acidic amino acids. 1 amino and 2 carboxyl groups.			
—	+	—	Hydroxy-glutamic acid.				
—	+	—	Aspartic acid.				

() Indicates that data are not available.

Tyrosine crystallises in tufts of fine, silky needles.

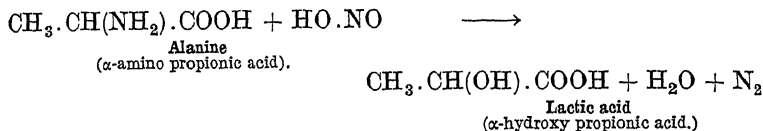
Leucine, which is usually closely associated with tyrosine, forms pearly plates and "cones."

By extracting the concentrated mixture with butyl alcohol, Dakin has been able to recover 90 per cent. of the amino nitrogen of hydrolysed gelatin in the form of amino acids.

The further separation of the acids requires special methods for each species.

General Reactions of the Amino Acids.—(1) *Deamination.*—When an amino acid is treated with nitrous acid, the α -amino group is decomposed and replaced by hydroxyl, the nitrogen escaping as gas, which can be measured.

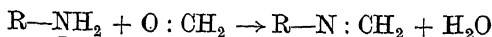
When an amino acid is deaminated in this way the corresponding hydroxy acid is produced :—



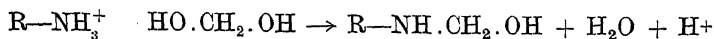
In Van Slyke's method, the amino compound is added to a mixture of 30 per cent. sodium nitrite and glacial acetic acid contained in a special type of apparatus, which is shaken vigorously for five minutes. The evolved gas is expelled, treated with alkaline permanganate to absorb nitric oxide, which forms as a by-product, and the residual nitrogen estimated in a manometer. Under standard conditions, the α -amino groups lose all their nitrogen within five minutes.

Nitrous acid will readily attack the α -amino group of an amino acid, but not other amino groups, such as the terminal or ω -amino group of lysine, citrulline and arginine. The explanation may be because the acid only reacts with the amino group when in the $-\text{NH}_3^+$ form. Urea, guanidine, creatine, and related compounds only react with nitrous acid when in presence of a strong acid, such as HCl, that is able to unmask and ionise the $-\text{NH}_2$ groups.

(2) *Formaldehyde Condensation.*—When an amino acid in neutral solution is mixed with excess of neutral formaldehyde solution, the mixture becomes acid, and can be titrated (Sørensen's reaction), thus affording an important method for the estimation of amino acids and ammonium salts. The mechanism of the change has been studied by Levy (1937). According to the older view, formaldehyde condenses with the amino group to form an unstable methylene-imino derivative, thereby destroying the basicity of the group.

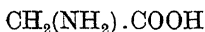


The more recent view is that an alcohol is formed, and the proton in union with the $-\text{NH}_2$ is released. Consequently, the method is really an estimation of amino groups present as $-\text{NH}_3^+$, as occurs in the dipolar amino acid molecule. The aldehyde reacts in hydrated form.



Subsequently, a second molecule of formaldehyde reacts with the remaining H atom in the —NH— system.

(3) *Decarboxylation*.—When salts of amino acids are heated or when they are attacked by the enzyme *carboxylase*, secreted by many moulds and bacteria, carbon dioxide is liberated, and the *amine* corresponding to the original amino acid is formed :—



Glycine.

Methylamine.

Many of these amines, notably *tyramine* from tyrosine, and *histamine* from histidine, are much more physiologically active than the parent amino acid, and their liberation is an important factor in surgical shock and similar conditions.

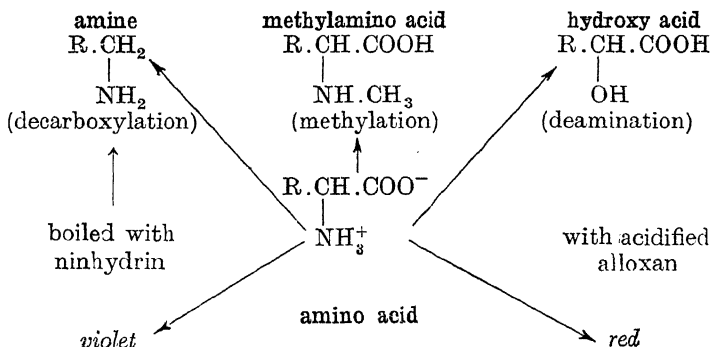
(4) *Colour Reactions*.—(a) *Ninhydrin Test*.—When a neutral solution of an α -amino acid is boiled with a few drops of a 0.2 per

cent. aqueous solution of ninhydrin, $\text{C}_6\text{H}_4 \begin{array}{c} \diagup \text{CO} \\ \diagdown \text{CO} \end{array} \text{C}(\text{OH})_2$, a violet

colour develops. Proteins and their intermediate cleavage products also give the test, ammonium salts give a purple colour. The test is negative with many important biological aminoid compounds, including urea, allantoin, uric acid, guanine, glucosamine, proline, and hydroxyproline.

(b) *Alloxan Test* (Denigès).—In dilute (1 per cent.) solution glycine, alanine and other simple amino acids develop a red colour when heated with about one-third their volume of a reagent containing 2 per cent. alloxan in 5 per cent. nitric acid. The test is not given by amino acids bound in protein form.

SUMMARY OF AMINO ACID REACTIONS



Amino Acid Percentage Content of Typical Proteins

Amino Acid.	Gelatin.	Casein.	Milk Albumin.	Egg Albumin.	Gliadin.	Zein.	Edestin.
Glycine	25.2	0.4	0.4	1.7	0.0	0.0	3.8
Alanine	8.7	1.8	2.4	2.2	2.0	9.8	3.6
Valine	0.0	7.9	3.3	2.5	3.3	1.9	+
Leucine + Iso-leucine . .	7.1	9.7	14.0	10.7	6.6	25.0	20.9
Aspartic acid	3.4	4.1	9.3	6.2	0.8	1.8	10.2
Glutamic acid	5.8	21.8	12.9	13.3	43.7	31.3	19.2
Hydroxy glutamic acid . .	0.0	10.5	10.0	1.4	2.4	2.5	—
Serine	0.4	0.5	1.8	—	0.1	1.0	0.3
Proline	9.5	8.0	3.8	4.2	13.2	9.0	4.1
Hydroxyproline	14.1	0.3	—	—	—	—	2.0
Phenyl alanine	1.4	3.9	1.2	5.1	2.3	7.6	3.1
Tyrosine	0.01	6.5	1.9	3.2	3.1	5.9	4.5
Cystine	0.1	0.3	4.0	1.3	2.4	0.8	1.0
Arginine	9.1	5.2	3.0	6.0	3.2	1.8	15.8
Histidine	0.9	2.6	1.5	1.4	2.1	1.2	2.1
Lysine	5.9	7.6	8.4	3.8	0.6	0.0	2.2
Tryptophane	0.0	2.2	2.7	1.3	0.8	0.17	1.5
Ammonia	0.4	1.6	1.3	1.3	5.2	3.6	2.3
Total	92.4	94.8	81.9	63.2	91.8	103.4	96.6

Values enclosed in a square denote that the protein is characterised by a high content of that particular acid.

The Molecular Weight and Dimensions of the Protein Molecule.—

Proteins and their cleavage products down to the smaller polypeptides form colloidal solutions in water; this implies that they are compounds of much greater molecular size and weight than, say, the simple sugars and the amino acids, and the ordinary thermal methods used for determining molecular weights are inapplicable, since colloids undergo changes when boiled or frozen in solution. The methods employed are (i.) *stoichiometric*, according to which the molecular weight is deduced from the content of a particular amino acid or element such as iodine, sulphur or iron, present in the molecule, on the assumption that the protein contains a simple multiple of the unit; (ii.) *physical*, depending on measurements of osmotic pressure, sedimentation equilibrium and sedimentation rate. The last two of these have been extensively studied by Svedberg, using an ultra centrifuge.

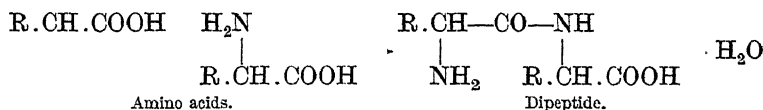
Sedimentation data obtained by Svedberg indicate that proteins fall into two classes: (i.) those with a radius between 2 $m\mu$ and 4 $m\mu$, and a molecular weight that is a simple multiple of 35,000;

and (ii.) those with a radius of $12\text{ m}\mu$, and upwards, and a molecular weight that is approximately a multiple of 400,000.

Examples of class (i.) are : lactoglobulin (37,800), pepsin (37,000), insulin (38,000), ovalbumin (40,500), hæmoglobin (68,000), serum globulin (150,000). Examples of class (ii.) are the hæmocyanins, one of which in the snail has a value of about 5,000,000.

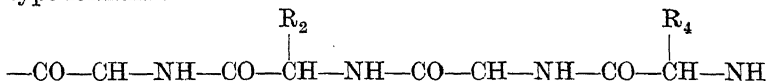
By X-ray analysis, Astbury and his colleagues have shown that proteins are composed of repeating structural units, the length and position of which may be measured. These observations confirm the distinction recognised between *fibrous* non-crystalline proteins, composing silk, hair, muscular and connective tissue, and *globular* crystalline proteins, such as egg albumin, hæmoglobin and insulin.

Structure of the Protein Molecule.—Since proteins are assembled from amino acid units, the simplest structure they can have is that of a polypeptide chain, which may be fragmented into smaller peptides. A dipeptide is a compound formed by condensation between the carboxyl group of an amino acid with the amino group of another acid. A tripeptide is formed by addition of an amino acid to a dipeptide.



Glycyl-glycine, $\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{COOH}$, is the simplest peptide.

(1) **The Peptide Theory** (Hofmeister and Emil Fischer).—The protein molecule is a chain composed of peptide links, and has the type formula :—



R represents various monovalent groups, twenty-two of which are now known, which form side-chains to a common main-chain.

Evidence in Support of the Peptide Theory.—(i.) All proteins give a violet or rose colour with copper salts in alkaline solution (the copper protein test), which is characteristic of the peptide linkage $=\text{CH}-\text{CO}-\text{NH}-\text{CH}=$, and not given by any individual amino acid other than serine, threonine and histidine, which have a somewhat similar arrangement in their molecules.

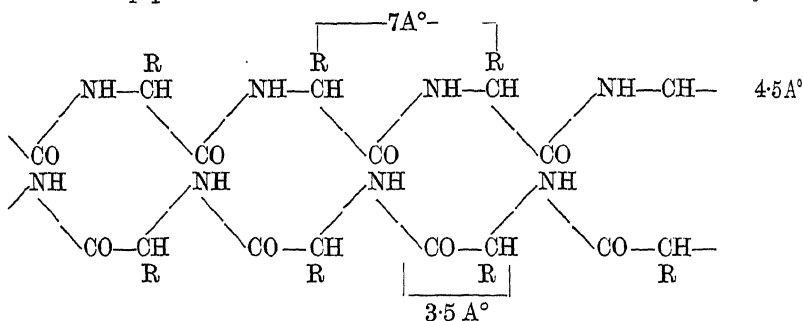
(ii.) During protein hydrolysis there is little change in the reaction.

The basic amino and the acid carboxyl groups are exposed at an equal rate, showing that they were originally present in combination.

(iii.) Natural proteins, other than protamines and histones, are neutral compounds, and the free amino groups that they display have been shown to be the terminal groups of lysine or arginine.

(iv.) Peptides of known constitution have been synthesised, some of which are hydrolysed by the proteoclastic enzymes in a manner comparable to the hydrolysis of natural proteins.

Developments of the Peptide Theory.—(a) *Lattice Structure.*—When a crystal is irradiated by X-rays, secondary rays are reflected from each surface within the structure, and form interference patterns that can be photographed. From a study of these patterns, Astbury concludes that proteins have a lattice structure owing to transverse association between alternate :CO and :NH groups in the peptide chain.



The average thickness of an amino acid residue in the chain is about 4.5 Å ($0.45\text{ m}\mu$), and its length is about 3.5 Å .

Hair is characterised by the appearance of a new type of X-ray pattern when it is stretched, showing that the keratin chains occur normally in a folded state. Hair protein resembles muscle protein that has been "vulcanised," or impregnated with sulphur, to decrease plasticity and increase resistance to wear (Astbury, 1936).

(b) *Pattern Frequency.*—From a study of the amino acid ratios in proteins, and the composition of derived peptides, Bergmann and Niemann conclude that the amino residues are arranged in regular order and frequency in the molecule. For example, silk fibroin has the pattern :—

—glycine—alanine—glycine—*X*—glycine—alanine—glycine—tyrosine—,

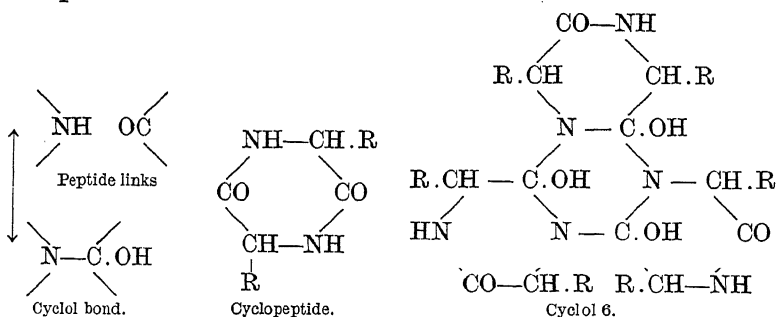
where *X* represents an unidentified unit.

In egg albumin, the amino acids occur in the following frequencies : glutamic acid, 1 in 8 ; aspartic acid, 1 in 18 ; methio-

nine, lysine and arginine, 1 in 24; tyrosine, 1 in 36; histidine and cysteine, 1 in 72. The smallest number containing these ratios is 288, hence the molecular weight of egg albumin must be some multiple of 288. A molecule of 288 residues has a molecular weight of 288 multiplied by the average weight of the residues, which gives a value of 35,700, a result of the same order as that obtained for egg albumin (40,500) by means of Svedberg's ultra-centrifuge.

(2) **The Cyclol Theory.**—Protein molecules are composed largely, if not entirely, of amino acid residues, and contain many —NH—CO— linkages but few free —NH_2 groups, apart from those found in side-chains. The general uniformity of the protein type suggests a uniformity of general structure. Many proteins are easily denatured, and when spread on a suitable surface spontaneously form insoluble monolayers.

Globular proteins in alkaline solution tend to aggregate. Protein crystals show a high trigonal symmetry. These and other observations have led Wrinch (1936) to discard the peptide chain theory in favour of a structure in which the unit is a cyclol made up of six amino acid residues. Each cyclol is further stabilised by cross union between a peptide nitrogen and an adjacent carbon atom, the result being called a "cyclol 6," which reoccurs throughout the protein fabric.



By folding a sufficient number of cyclols it is possible to make a closed cage, which according to Wrinch, represents the protein molecule.

These cages are in the form of hollow truncated tetrahedra, constructed from a simple multiple of 72 amino acid units, the R radicles of which either project into the cavity or extend outwards from the surfaces. Wrinch suggests that the globular molecules of insulin, pepsin and egg albumin contain 72×4 amino acid units, which conforms with Svedberg's data for dimension

and density, and with the conclusions of Bergmann, but awaits confirmation by X-ray analysis. Evidence against the theory is discussed by Pauling and Niemann (1939).

ANALYTICAL REACTIONS OF PROTEINS

A. General Colour Reactions given by all Proteins.—These depend on characteristic amino acid groupings in the protein molecule.

(1) **The Protein Copper Test**, or “Biuret” Test.—To 5 ml. of solution add 1 ml. of 20 per cent. NaOH. Then add 1 or 2 drops of dilute (1 per cent.) CuSO_4 . If a protein be present the solution becomes purple. Using the minimal quantity of copper sulphate, it is possible to distinguish two shades of colour :—

- (i.) *Violet*, given by all higher proteins and also by gelatin.
- (ii.) *Rose-pink*, given by all lower proteins (proteoses, peptones, peptides), except gelatin. Excess of copper renders these shades indistinguishable.

Acidify the mixture with 20 per cent. acetic acid until the colour is discharged. A precipitate forms if the solution contains a higher protein, not if it contains a lower protein.

The colour depends on the presence of the peptide linkage $=\text{CH}-\text{CO}-\text{NH}-\text{CH}=\text{}$ in the protein molecule. A somewhat similar colour is given by other compounds containing this grouping, such as *biuret*, $\text{H}_2\text{N}-\text{CO}-\text{NH}-\text{CO}-\text{NH}_2$, hence the older and misleading name for the test. Proteins do not contain biuret.

Acidification is of use in distinguishing between a doubtful violet or rose colour. Proteins (except gelatin) that give a violet copper reaction are precipitated on subsequent acidification of the mixture. No protein giving a rose colour is precipitated on subsequent acidification.

Ammonium salts inhibit the test by forming a deep blue colour with the CuSO_4 ; this may be overcome by addition of a large excess of NaOH, but it is a common source of error in testing filtrates after saturation with ammonium sulphate.

(2) **The Arginine Test.**—Make 5 ml. of solution alkaline with 5 drops of 20 per cent. NaOH. Add 5 drops of 2 per cent. alcoholic α -naphthol, followed by 5–10 drops of 2 per cent. sodium hypochlorite (NaOCl) or 1–2 drops of bromine water. An intense carmine develops if arginine or a protein containing arginine be present. Arginine appears to be a universal constituent of proteins, and the reaction may be regarded as a general protein test.

The colour depends on the presence of the monosubstituted *guanidine grouping*, $\text{H}_2\text{N}-\text{C}(\text{:NH})-\text{NH}\cdot\text{R}$, in the molecule.

(3) **Ninhydrin**, the general reagent for amino acids (p. 143), also reacts with proteins in slightly alkaline solution to give violet colours.

B. Colour Tests due to Individual Amino Acids

(1) **Xanthoprotein Test.**—Add 5 drops of concentrated nitric acid to about 3–5 ml. of protein solution. A precipitate forms if higher proteins be present (Heller's test). Heat the mixture. A yellow colour develops if the test be positive. Cool, and make alkaline with ammonium hydroxide. The colour deepens to orange.

The test is due to the presence of substituted benzene rings occurring as tyrosine and tryptophane in the protein. It is given also by free tyrosine, tryptophane and other benzene derivatives.

Gelatin is the commonest protein that does not give the xanthoprotein test owing to its lack of the necessary amino acids.

(2) **Tyrosine Test.**—Add 5 drops of 1 per cent. mercuric sulphate in 10 per cent. sulphuric acid to 5 ml. of protein solution. A precipitate forms with most proteins. Heat the mixture and add a drop of 1 per cent. sodium nitrite. A red colour develops if tyrosine be present.

This is Cole's modification of the original test, in which Millon's reagent is used, unaccompanied by sodium nitrite.

The test is given by many phenolic compounds, of which tyrosine is the only one found in proteins.

Chlorides and excess of alkali inhibit the test by precipitating the mercury. This can be compensated by addition of more reagent.

Pure gelatin with a tyrosine content of about 0.01 per cent. gives a negative result with the test when in solutions of less than 20 per cent. concentration.

(3) **Tryptophane Tests.**—(i.) Add 3 drops of Ehrlich's aldehyde reagent to about 2 ml. of protein solution, followed by excess (3–5 ml.) of concentrated hydrochloric acid. Heat gently to boiling. A violet colour develops if the protein contains tryptophane. Add 1–3 drops of 0.1 per cent. hydrogen peroxide. The colour changes to deep blue. Excess of the oxidiser rapidly bleaches the pigment.

This test is very sensitive, and will detect 1 part of tryptophane in 500,000.

The reagent is 2 per cent. *p*-dimethylamino-benzaldehyde in alcohol or 5 per cent. hydrochloric acid. It gives characteristic colours with hexosamines (p. 99), indole and scatole (p. 310), urobilinogen (p. 186), urea (p. 384), allantoin (p. 347), and indoxyl (p. 400). Tryptophane appears to be the only biological compound that gives a deep blue colour with the reagent.

(ii.) Mix 3 ml. of solution with about 2 ml. of the Hopkins-Cole reagent or with 2 ml. of glacial acetic acid that has been "activated" by exposure to sunlight. Then add slowly about

3 ml. of concentrated sulphuric acid so as to form a layer below. A deep purple ring develops at the junction of the liquids if tryptophane be present.

The active ingredient in both reagent and acetic acid is glyoxylic acid, $\text{HOOC}\cdot\text{CHO}$, the recognition of which led to the subsequent discovery of tryptophane by Hopkins and Cole.

Most proteins, with the exception of gelatin and zein, give a positive result with the tryptophane tests.

(4) **Cystine Test.**—Add a few drops of 5 per cent. lead acetate to 5 ml. of protein solution, followed by sufficient 20 per cent. sodium hydroxide to redissolve the white precipitate of lead hydroxide that first forms. Boil the mixture. If cystine or proteins containing cystine be present, the solution turns dark brown owing to formation of lead sulphide.

(5) **Thiol Test.**—Mix equal volumes (2–3 ml.) of protein solution and saturated ammonium sulphate. Add 2–4 drops of fresh 5 per cent. sodium nitroprusside, and make alkaline with a few drops of ammonium hydroxide. If metaprotein, glutathione, cysteine, or other compounds containing the thiol group, $-\text{SH}$, be present, a purple colour develops.

Heat-coagulation or acid-denaturation of higher proteins unmasks thiol groups. During subsequent hydrolysis these groups tend to disappear, perhaps by oxidation to dithio groups, $-\text{S}-\text{S}-$, such as occur in cystine, which does not give the test.

A somewhat similar colour reaction is given by acetone and acetic acid (Rothera's test, p. 407).

(6) **Citrulline Test.**—Add 3 drops of 3 per cent. diacetyl monoxime to 2 ml. protein solution. Add about 3 ml. of concentrated hydrochloric acid, or sufficient to redissolve any protein that may be precipitated. Heat to boiling for half a minute. Allow to cool, and carefully oxidise by addition of 1–2 drops of very dilute (0.1 per cent.) hydrogen peroxide or 1 per cent. potassium persulphate.

If the test be positive a carmine colour gradually develops, and may be intensified by heating. Excess of oxidiser rapidly bleaches the pigment.

The test is given by urea and substituted ureas, of which citrulline is the only example known to occur in ordinary proteins.

Prolonged boiling must be avoided. Otherwise, extraneous colour reactions may arise from carbohydrate and tryptophane present in many proteins.

In alkaline solutions, free diacetyl reacts with arginine and with creatine, and other substituted guanidines to give red pigments (Harden's test).

Group Tests for Proteins

(1) Heat-Coagulation (Albumins and Globulins)

Acidify 5 ml. of solution with 1–3 drops of 20 per cent. acetic acid. Add a drop of 5–10 per cent. sodium chloride. Boil gently. A white coagulum shows the presence of albumin or globulin. This test is of special importance in the detection of these proteins in urine.

(2) Fractional Precipitation by Neutral Salts

(a) *Half-saturation*.—Mix 5 ml. of solution with an equal volume or slightly more, of saturated ammonium sulphate.

A white precipitate is given by globulins, caseinogen and other higher proteins, with the notable exception of albumins.

(b) *Full-saturation*.—Filter the mixture after half-saturation and add 10 ml. of the filtrate to a test tube containing about one-third its volume of solid ammonium sulphate. Mix well. All higher proteins and lower proteins, except peptones and peptides, are precipitated, as is shown by the appearance of a turbidity in the liquid above the layer of undissolved ammonium sulphate.

Fractional precipitation, or “salting-out,” is used in the separation and identification of mixed proteins. Unlike heat-coagulation, it is a reversible process, and the protein precipitate can be redissolved on addition of water or removal of the salt by dialysis.

MgSO_4 , Na_2SO_4 and NaCl are also used as neutral precipitants but are not as effective as $(\text{NH}_4)_2\text{SO}_4$ or ZnSO_4 .

(3) Precipitation by Special Reagents

Acids, Metals, and Alkaloidal Reagents.—Concentrated strong acids, notably nitric and trichloroacetic, CCl_3COOH , precipitate most proteins higher than peptones; concentrated tannic acid precipitates peptones as well as higher proteins.

Heller's Test, which is important in the detection of albumin in urine, is carried out by placing about 2 ml. of concentrated nitric acid in a test tube, and carefully adding about 5 ml. of solution so as to form a layer on top. If a higher protein be present a white, cloudy ring forms at the junction of the liquids.

The Salicylsulphonic Acid Test.—A few drops of a 20 per cent. solution of this acid will give a white precipitate with higher proteins even in very dilute solutions. Peptones are not precipitated.

Proteins, especially in acid solution, are precipitated by many of the “heavy metals,” including silver, mercury, lead, and copper. For this reason, egg-white is given as an antidote in poisoning by these metals.

ESTIMATION OF PROTEINS

(1) **Total Nitrogen Determinations.**—Since proteins are the outstanding nitrogen compounds of the organism they are often estimated in tissues and precipitates by determining the total nitrogen, and multiplying the result by a factor, usually 6.5, since proteins contain about 15–16 per cent. of nitrogen. The result gives the protein percentage in the material.

The total nitrogen is found by Kjeldahl's method, in which a known weight of material is boiled with concentrated sulphuric acid until all its nitrogen has been fixed as ammonium sulphate. Excess of sodium hydroxide is then added to liberate the ammonia, which is distilled over into a known volume of standard acid. From the amount of acid neutralised, the volume of liberated ammonia can be calculated and expressed in terms of nitrogen.

(2) **Amino Nitrogen Determinations.**—In this method, developed by Van Slyke, the mixture is treated with nitrous acid in a special apparatus. Each molecule of nitrogen gas set free corresponds to one molecule of nitrous acid and one α -amino group, and from this the total number of such groups present is calculated.

(3) **Decarboxylation** occurs when free amino acids are boiled with ninhydrin, and the CO_2 evolved can be measured. Van Slyke and Dillon (1938) regard this as the most specific method for estimating amino acids.

These methods are adapted for following the progress of protein hydrolysis, and for estimating the protein and amino acid content of the various fractions.

(4) **Colorimetric Methods.**—Many of the protein colour tests can be made quantitative by comparing the intensity of the colour with a standard test containing a known amount of the same protein.

(5) **Precipitation and Coagulation Methods.**—Albumin and globulin can be estimated absolutely by weighing the dry residue after heat coagulation and filtration. They and other higher proteins can also be estimated approximately by picric acid precipitation (Esbach's method), the volume of the precipitate being read after the mixture has stood for twelve hours in a graduated tube.

The cloudy precipitate formed on addition of salicylsulphonic acid may be matched in a comparator against a series of standard precipitates. This method is now employed in the estimation of protein in urine; it is an example of *nephelometry* or measurement of opalescence.

Percentage Distribution of Protein in Natural Sources

Blood, human : Total protein, 18-21 ; hæmoglobin, 13-16.
 Plasma, human : Total protein, 7-8 ; seralbumin, 4-6 ; serglobulin, 1-2.3 ; fibrinogen, 0.3-0.6.
 Milk, human : Total protein, 1-2.6 ; caseinogen, 1-1.3 ; lactalbumin, 1-1.5.
 Milk, cow : Total protein, 2.6-3.5 ; caseinogen, 2.5-3 ; lactalbumin, 0.5.
 Muscle, human : Total protein, 17-19 ; myosin, 12-15 ; myogen, 4-6.
 Egg white, hen : Total protein, 10-13 ; ovalbumin, 9-11 ; ovoglobulin, 0.7 ; ovomucoid, 1.
 Egg yolk, hen : Total protein, 15-16 ; vitellin, 11-13 ; livetin, 3-4.

Summary of the Reactions of the Commoner Proteins

A. Colour Tests

Protein.	Copper Test.		Xantho-protein.	Tyrosine.	Tryptophane.	Arginine.	Citrulline.
		+ acid.					
Albumin .	violet	ppt.	+	+	+	+	+
Globulin .	violet	ppt.	+	+	+	+	+
Metaprotein .	violet	ppt.	+	+	+	+	+
Proteose .	rose	no ppt.	+	+	+	+	+
Peptone .	rose	no ppt.	+	+	+	+	+
Caseinogen .	violet	ppt.	+	+	+	+	+
Gelatin .	violet	no ppt.	—	—	—	+	(+)

B. Precipitation Tests

Protein.	Heat-coagulation.	Half-saturation with $(\text{NH}_4)_2\text{SO}_4$.	Full-saturation with $(\text{NH}_4)_2\text{SO}_4$.
Albumin . .	coagulates	not precipitated	precipitated
Globulin . .	coagulates	precipitated	precipitated
Metaprotein . .	precipitates at pH6, and then coagulates if heated at pH6.	precipitated	precipitated
Proteose . .	non-coagulable	not precipitated	precipitated
Peptone . .	non-coagulable	not precipitated	not precipitated
Gelatin . .	non-coagulable	precipitated	precipitated
Caseinogen . .	non-coagulable	precipitated	precipitated

Qualitative Analysis of Protein Mixtures

Milk. (1) **Separation of Caseinogen by Acidification.**—Dilute 20 ml. of milk with 40 ml. of water. Add 1 ml. (20 drops) of 20 per cent. acetic acid, drop by drop, stirring at intervals. The chief protein of cow's milk is caseinogen. It separates out from a slightly acid (pH 4.6) solution as a flocculent precipitate carrying with it the milk fat. Filter, and re-filter if the filtrate is not quite clear. Excess of acid redissolves some of the caseinogen, and causes the filtrate to be turbid.

Collect the residue, wash with water, and suspend in about 10 ml. of water. Apply the general and special protein colour tests. All of them are given by caseinogen, but the cystine test may be indefinite. The filtrate contains the heat-coagulable proteins lactalbumin and lactoglobulin, as can be shown by applying the colour tests before and after heat-coagulation, followed by filtration. In human milk the chief protein is lactalbumin.

(2) **Separation of Caseinogen by Rennin.**—To 5 ml. of milk add 5–10 drops of an active preparation of the enzyme *rennin*, obtained from the gastric mucosa of calves. Mix and incubate at 40°. In a few minutes the milk will solidify owing to the conversion of caseinogen into insoluble casein (paracasein), and the tube may be inverted without spilling the contents.

After keeping the mixture for about an hour, the casein clot will have contracted, leaving a clear layer of milk serum, or whey, on top. The casein may be removed by filtration, and the filtrate tested for lactalbumin and globulin as before.

Albumin and Globulin. Products of Hydrolysis.—When a higher protein is hydrolysed by acids or enzymes a series of products is obtained.

<i>Protein</i>	albumin + globulin	→ metaprotein → proteose → peptone → peptides →	amino acids
<i>Copper test</i>	violet	- rose-pink -	- — — — no change

From the examination of the products of the gastric digestion of albumin it is possible to determine the efficiency of the digestive process during a given interval of time.

Identification of the Products of Hydrolysis.

- (1) Apply the copper-protein test to 20 ml. of the mixture.
If negative, no unhydrolysed protein is present.
If positive, higher and/or lower protein is present.
- (2) Acidify the copper mixture with 20 per cent. acetic acid.

Precipitate.

Higher protein present.

(a) Filter. Make filtrate alkaline with 20 per cent. sodium hydroxide so as to regenerate the copper test.

If positive, lower protein is present as well as the higher protein previously removed.

(b) Confirm presence of higher protein by applying heat-coagulation test to original solution.

Filter. Confirm presence or absence of lower protein by applying tests (c) and (d) to filtrate.

No precipitate.

Lower protein only present.

(c) Identify lower protein by full saturation of original solution with ammonia sulphate.

Precipitate = proteose.

(d) Filter, and test for protein in filtrate by xanthoprotein test.

If positive, peptone or peptides are present.

Distinction between Albumin and Globulin.—Half-saturate 20 ml. of the solution with ammonium sulphate. Any globulin present will be precipitated. Filter. Apply the heat-coagulation test to the filtrate. If positive, albumin is present.

Identification of an Unknown Protein.—(a) *In solution.* Apply the tests in the following order :—

- (1) Copper-protein test followed by acidification. This distinguishes between higher and lower protein.
- (2) Heat-coagulation test. This shows presence or absence of albumin and globulin.
- (3) Xanthoprotein, tyrosine and tryptophane tests. If these are negative, the protein is possibly gelatin or zein.
- (4) Rennin test. If positive, the protein is caseinogen.

(b) *In Solid Form.* Dissolve if possible in water or weak alkalis, and apply tests in above order to the solution. If the protein will not dissolve it is probably casein or heat-coagulated protein. Apply the colour tests directly to the solid material.

DETECTION OF PROTEINS IN SIMPLE MIXTURES

Copper-protein Test

Purple	No change
= Protein	= No protein

Acidify with 20 per cent. Acetic Acid

Precipitate	No precipitate
= Higher protein	= Lower protein

Proteose Peptone	Tyrosine and tryptophane tests positive
---------------------	--

Filter	Gelatin	Tyrosine and tryptophane tests negative
--------	---------	--

Residue	Filtrate
= Higher protein	May contain lower protein

Identify by testing
original solutionMake alkaline with Sodium Hydroxide

Purple colour restored	No purple colour
= Lower protein	= No protein

Albumin	Coagulated by heat
Globulin	
Metaprotein	Thiol nitroprusside test positive
Caseinogen	Coagulated by rennin.

This scheme of analysis is useful for the examination of artificial mixtures and peptic and tryptic digestion products. It does not differentiate between albumin and globulin or between proteose and peptone when present along with other higher and lower proteins. Such differentiation can be accomplished best by fractional precipitation with neutral salts, supplemented by the foregoing tests.

GENERAL REFERENCES (Chapters VIII and IX)

- ASTBURY, W. T. (1939), "X-Ray study of proteins." *Science Progress*, **34**, 1.
- N, M. (1938), "Structure of proteins in relation to biological problems." *Chem. Rev.*, **22**, 423.
- CLARKE, H. T. (1938), "Natural amino acids." *Organic Chemistry*, Ed. Gilman, **2**, 859.
- COHN, E. J. (1935), "Chemistry of the proteins and amino acids." *Ann. Rev. Biochem.*, **4**, 93.
- ION, W. R. (1939), "The carbamido-diacetyl reaction." *Biochem. J.*, **33**, 902.
- ORTNER, R. A. (1938), "Outlines of Biochemistry." 2nd Ed. London.
- KLARMANN, E. (1927), "Recent advances in the determination of the structure of the amino acids." *Chem. Rev.*, **4**, 51.

- KOSSEL, A. (1928), "Protamines and Histones." London.
- LLOYD, D. J., and A. SHORE (1938), "Chemistry of the Proteins." London.
- MITCHELL, H. H., and T. S. HAMILTON (1929), "Biochemistry of the Amino Acids." London.
- PIETTRE, M. (1937), "Biochimie des Protéines." Paris.
- RIMMINGTON, C. (1936), "Chemistry of the proteins and amino acids." *Ann. Rev. Biochem.*, **5**, 117.
- SCHMIDT, C. L. (1938), "Chemistry of the Amino Acids and Proteins." London.
- VICKERY, H. B., and C. L. SCHMIDT (1931), "History of the discovery of the amino acids." *Chem. Rev.*, **9**, 169.

CHAPTER X

LIPIDES

LIVING organisms are not soluble in water ; they may be drowned, but they are not dissolved. Resistance is conferred partly by the use of protective coverings of scleroproteins on the surface of the animal body, and partly by the presence of water-insoluble compounds incorporated into the tissues and cell membranes. Many of these compounds are fats or fat-like substances, and can be extracted from dried animal and plant tissues by appropriate organic solvents. All these compounds are soluble in ether, and in this respect differ sharply from the carbohydrates and the proteins, and constitute the third great family of bio-organic compounds, namely, the **lipides**.

Definition.—Lipides are esters of higher aliphatic acids obtained from living tissues and characterised by insolubility in water, and solubility in the “fat solvents” : ether, chloroform, carbon tetrachloride, light petroleum (petrol), benzene, xylene, hot alcohol and hot acetone.

Distribution.—Lipides are universally distributed, partly as structural lipid or “tissue fat” in all cells, especially those of the nervous system, and partly as storage lipid, or “reserve or depot fat” in adipose tissue, seeds rich in oil, and other specialised regions. The distinction is physiological ; storage lipid is used in auto-nutrition, structural lipid is not.

Nomenclature.—Lipides may be divided into :—

<i>Simple Lipides.</i>	<i>Complex Lipides.</i>
(The fat group).	(The lipine group).
Contain only C, H, and O.	Contains C, H, O, N, and often
Mostly found as storage lipid.	P and S.
Usually simple glycerides of	Mostly found as structure lipid.
aliphatic acids.	Complex esters of various acids
	and bases.

In addition there are the *lipide derivatives*, products of hydrolysis, many of which are soluble in fat solvents.

There is an indefinite group of fat-soluble compounds, such as the carotene pigments, the terpenes, and the essential oils, which are removed along with the lipides during the process of extraction.

Although they are similar in regard to solubility they are quite distinct chemically and physiologically from either the fats or the lipines, and are excluded from the lipid family.

Classification of the Lipides.—A. *Simple lipides*, or *true fats*. Esters of higher aliphatic acids with various alcohols. Contain only C, H and O.

- (1) **Oils** ; glycerol esters liquid at 20° C.
- (2) **Fats** ; glycerol esters solid at 20° C.
- (3) **Waxes** ; esters of aliphatic acids and higher alcohols ; usually solid at ordinary temperature.

B. *Complex Lipides*, or *Lipines*.—Esters of aliphatic acids and complex alcohols. They contain C, H, O, N, and sometimes P and S.

- (1) **Phospholipides**, (phospholipines or phosphatides) : Lipines containing phosphoric acid and a nitrogenous base.

Examples are : *lecithin*, *cephalin*, *sphingomyelin*.

- (1A) **Phosphotidates** : Phospholipides from which the nitrogen base has been removed.

- (2) **Glycolipides** (galactosides or cerebrosides) : Esters of aliphatic acids and carbohydrates. They contain N but no P.

Examples are : *kerasin* and *phrenosin*.

- (3) **Aminolipides** : compounds of aliphatic acids and substituted amines. Example : *bregenin*. They contain no P.

- (4) **Sulpholipides** : Lipides containing sulphuric acid and a nitrogenous base. Example : *sulphatide* from brain.

C. *Lipide Derivatives*.—Products of hydrolysis.

- (1) **Lipide Acids**.—Higher members of the acetic series of acids and related acids of the unsaturated oleic, linoleic, and linolenic series.

- (2) **Lipide Alcohols**.—Glycerol (glycerin) and higher solid alcohols.

- (3) Miscellaneous bases.

Lipoids.—These are biological compounds resembling the lipides in certain physical properties, notably solubility in fats and fat-solvent. The term is sometimes extended so as to include the complex lipides.

Lipoids as a type are non-saponifiable, although some of the sterols may occur as esters with aliphatic acids. A systematic classification of the lipoids is unsatisfactory, since the only common property of these compounds is the physical one of solubility in certain organic solvents. Examples of biological lipoids occur in the essential or volatile oils of plants and the fat-soluble pigments (lipochromes) of plants and animals. In addition, some of the vitamins and the hormones belong to the lipid type, in that they are fat-soluble.

Important lipid families are :—

- (1) *Steroids*.—Derivatives of a four-ring pentanophenanthrene nucleus. Many steroids occur as esters of aliphatic acids, and for this reason are often included among the lipid derivatives.
- (2) *Carotenes*.—Orange or red hydrocarbon pigments derived from *ionone* (p. 202).
- (3) *Terpenes*.—Unsaturated hydrocarbons derived from *isoprene*, and found in the essential oils and oelo-resins of plants.

Unlike fatty oils, they are volatile in steam.

LIPIDE DERIVATIVES

All fats, aliphatic oils, and waxes belong to the ester class of compounds, and on hydrolysis liberate alcohol and acids. Hence, in studying the lipides it is convenient to start with a survey of these aliphatic or lipidic acids.

Aliphatic Acids.—At least eight different series of organic acids are represented in the natural lipides. One of these, the acetic series, is saturated ; the others are all more or less unsaturated.

(1) **The Acetic or Stearic Series, $C_nH_{2n+1}COOH$.** The chief members are :—

Acid.	Formula.	Solubility in H_2O .	Occurrence.
Formic .	$H.COOH$	Miscible	Stings of insects and plants.
Acetic .	$CH_3.COOH$	„	Vinegar.
Butyric .	$C_3H_7.COOH$	„	Butter fat.
Caproic .	$C_5H_{11}.COOH$	4 : 100 at $15^\circ C$.	Coconut oil.
Caprylic .	$C_7H_{15}.COOH$	9 : 1000 at $15^\circ C$.	Palm oil.
Capric .	$C_9H_{19}.COOH$	1 : 1000 at $100^\circ C$.	Laurel oil.
Myristic .	$C_{13}H_{27}.COOH$	Insoluble	Nutmeg oil.
Palmitic .	$C_{15}H_{31}.COOH$	„	Most animal and vegetable fats and oils.
Stearic .	$C_{17}H_{35}.COOH$	„	Most animal and vegetable solid fats.
Arachidic .	$C_{19}H_{39}.COOH$	„	Peanut (Arachis) oil.
Lignoceric .	$C_{23}H_{47}.COOH$	„	Glycolipides.
Cerotic .	$C_{25}H_{51}.COOH$	„	Beeswax.
Melissic .	$C_{29}H_{59}.COOH$	„	Beeswax.

A characteristic of the series is the non-occurrence in natural fats of fatty acids with an even number of carbon atoms in the residue attached to the carboxyl group, $COOH$.

A rare exception is porpoise oil, which contains an ester of valeric acid, $C_4H_9.COOH$.

Tuberculo-stearic (10-methyl stearic), $C_{18}H_{37}.COOH$ and phthioic acid, $C_{25}H_{51}COOH$, occur in the lipides of the human tubercle bacillus.

Palmitic Acid, $\text{CH}_3(\text{CH}_2)_{14}\cdot\text{COOH}$, m.p. 62°C ., occurs in many plant and animal fats and waxes, especially palm oil, Japan wax, and myrtle wax. It is present in spermaceti as cetyl palmitate and in beeswax as myricyl palmitate. The acid is insoluble in water, slightly soluble in cold alcohol, and easily soluble in hot or boiling alcohol.

Stearic acid, $\text{CH}_3(\text{CH}_2)_{16}\cdot\text{COOH}$, m.p. $69\text{--}32^\circ\text{C}$., is very widely distributed, especially in the body fat of higher animals as tallow, lard, and suet. It is prepared in quantity by the catalytic hydrogenation or "hardening" of the corresponding unsaturated acids, oleic, linoleic, linolenic, and clupadonic, all of which have the same number of carbon atoms. Stearic acid is a white solid, with a faint but characteristic smell. It is insoluble in water, but easily soluble in boiling alcohol.

(2) **The Oleic Series**, $\text{C}_n\text{H}_{2n-1}\cdot\text{COOH}$.—Unsaturated acids having one double bond, $-\text{CH}:\text{CH}-$. Fifteen members of the series occur naturally.

Oleic acid, $\text{C}_{17}\text{H}_{33}\cdot\text{COOH}$, m.p. 6°C ., is the most widely distributed of all the fatty acids. The free acid is a colourless liquid that turns yellow on exposure to air and light. It is a typical example of an *unsaturated* compound, and its presence in glycerides is shown by its property of reducing osmium tetroxide ("osmic acid"), OsO_4 , with the formation of a black deposit, easily recognised in histological preparations.

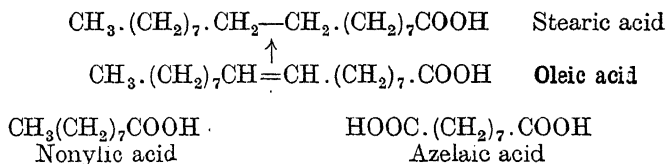
The Structure of Oleic Acid.—(i.) Combustion shows that the empirical formula is $\text{C}_9\text{H}_{17}\text{O}$.

(ii.) Molecular weight determination gives the formula, $\text{C}_{18}\text{H}_{34}\text{O}_2$.

(iii.) Titration shows that a single carboxyl group is present, $\text{C}_{17}\text{H}_{33}\cdot\text{COOH}$.

(iv.) On reduction, oleic acid takes up two atoms of hydrogen, and is converted into the corresponding saturated acid, *stearic acid*, $\text{C}_{17}\text{H}_{35}\text{COOH}$. This shows that there must be one double bond, or $-\text{CH}=\text{CH}-$ link, in the oleic acid molecule, and that the acid has a straight chain.

(v.) On oxidation, oleic acid breaks up into nonylic acid and azelaic acid, both of which are C_9 acids, indicating that the double bond in oleic acid is midway in the chain.



This shows that oleic acid is a $\Delta^{9:10}$ unsaturated acid; the suffix denoting that a double bond occurs between the ninth and tenth carbon atoms, as numbered from the right.

Other examples of the oleic series are: tiglic acid, C_4H_7COOH , from croton oil; and erucic acid, $C_{22}H_{43}.COOH$, from mustard oil.

Each member contains one double bond and is converted on reduction into a corresponding member of the stearic series.

(3) **The Linoleic Series**, $C_nH_{2n-3}.COOH$.—The natural acids are all C_{18} compounds, and were obtained first from linseed oil. They do not occur in human storage fat, but are found mostly as phospholipides and as esters of cholesterol. Linoleic acid, the chief member, is an important constituent of vegetable "drying oils"; on which it confers the power of hardening to form a film on exposure to the air. Its formula is $C_{17}H_{31}.COOH$.

It appears to be essential in nutrition, and is present in butter.

(4) **The Linolenic Series** is represented by linolenic acid, $C_{17}H_{29}.COOH$, which makes up about 20 per cent. of linseed oil, and occurs in all vegetable drying oils.

(5) **The Clupadonic Series** is represented by clupadonic acid, $C_{17}H_{27}.COOH$, which is found in cod-liver oil, and probably all marine oils; and arachidonic acid, $C_{19}H_{31}.COOH$, found in the lipoids, lecithin and cephalin.

(6) **Hydroxy acids** are represented by the **Cerebronic Series**, including phrenosinic or cerebronic acid from phrenosin of brain tissue; and the **Ricinoleic Series**, including ricinoleic acid, the glyceride of which is the chief constituent of castor oil, obtained from the seeds of *Ricinus communis*.

(7) **The Cyclic acids** include the two members, chaulmoogric acid from chaulmoogra oil and hydnocarpic acid from hydnocarpus and chaulmoogra. They are used in the treatment of leprosy.

Aliphatic Alcohols.—Natural fats and oils are esters of the trihydroxy alcohol glycerol; the other aliphatic alcohols occur either free as solutes in oils and fats or combined as *waxes*. Five groups of these lipidic alcohols may be recognised, four of which belong to the straight-chain type of compounds.

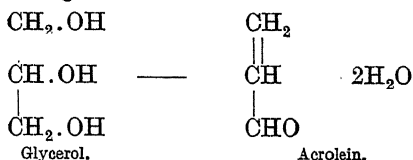
(1) Alcohols of the *Ethyl Alcohol Series*, $C_nH_{2n+1}.OH$; these contain one hydroxyl group. Being higher members of the series, they are all odourless and tasteless solids, insoluble in water but soluble in fat solvents. They are characteristic constituents of the waxes. The chief examples are: cetyl alcohol, $C_{16}H_{33}.OH$, from spermaceti; carnaubyl alcohol, $C_{24}H_{49}.OH$, from wool wax; melissyl, or myricyl alcohol, $C_{30}H_{61}.OH$, from beeswax.

- (2) Dihydroxy alcohols of the *Glycollic Series*, represented by coryphyl alcohol, $C_{24}H_{48}(OH)_2$, from carnauba wax.
- (3) Trihydroxy alcohol of the *Glycerol Series*. Only one member is known, glycerol, the basic constituent of all true fats.
- (4) Compound alcohols.
- (5) Sterols.

Glycerol, or glycerin, $CH_2OH.CH(OH).CH_2OH$, is quantitatively the most important of the lipid alcohols, and is obtained during the saponification of fats and oils. It is a colourless, odourless, viscid liquid, with a sweet, pungent taste. It is a good solvent, and is very hygroscopic, absorbing about 50 per cent. of its weight of water from the atmosphere. For this reason it is used in cosmetics and "vanishing creams" designed to keep the skin moist and sticky.

Free glycerol is not a lipid. It is insoluble in most of the fat solvents, and is miscible in all proportions with water and with alcohol.

Reactions of Glycerol.—(1) *Dehydration*.—On being heated rapidly along with an anhydrous salt, such as $NaHSO_4$, glycerol loses two molecules of water, and is dehydrated to form the unsaturated aldehyde acrolein, which imparts the characteristic acid smell to burning fat.



(2) *Esterification*.—Glycerophosphoric acid, $CH_2OH.CH(OH).CH_2O.PO:(OH)_2$, is prepared by heating glycerol in 60 per cent. phosphoric acid at $100^\circ C$. It is a characteristic constituent of the *phosphatides*, such as lecithin.

(3) *Colour Test*.—An aqueous solution of glycerol gives a blue colour on addition of a few drops of 5 per cent. potassium chromate and excess of nitric acid, showing the presence of the primary and secondary alcohol groups (p. 109).

Nitroglycerin, glyceryl trinitrate, $CH_2(O.NO_2).CH(O.NO_2).CH_2(O.NO_2)$, is prepared by adding glycerol to a cooled mixture of nitric and sulphuric acids. It is an ingredient in many high explosives, and is also used therapeutically as a general vaso-dilator.

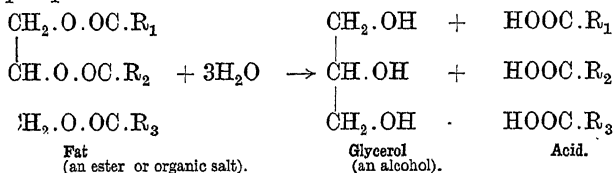
Compound alcohols or alcohol ethers occur in fish-liver oils, for example, *batyl alcohol*, $C_{21}H_{42}O(OH)_2$, a monoglycerol ester of octadecyl alcohol.

Sterols, or Alcohols of the Cholane Series.—These differ from the other lipid alcohols in the possession of a complex

cyclic structure. They are represented by the zoosterols, including *cholesterol*, $C_{27}H_{45}.OH$, found in animals, and the *phytosterols* and *zymosterols*, found in plants.

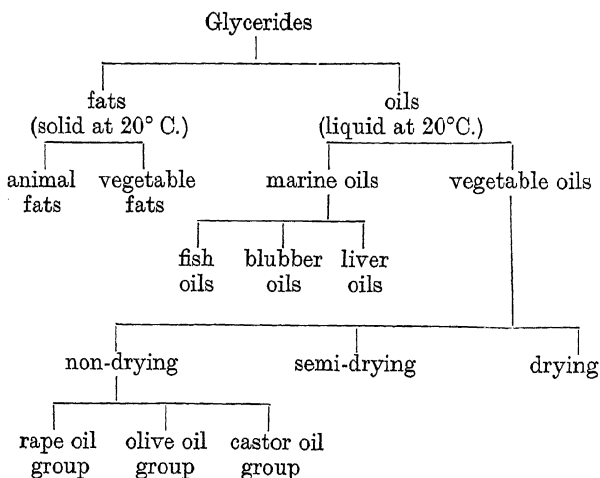
OILS AND FATS

These are *glycerides*, that is to say, esters of the trihydroxy alcohol, glycerol, and three molecules of aliphatic acid. On hydrolysis all are resolved into their constituents in accordance with the type equation :



The glycerol may be united to three molecules of the same acid, or three molecules of different acids, the latter constituting a mixed glyceride. Hydrolysis may be accomplished by enzymes, such as the lipases of the alimentary tract, by alkalies, or by superheated steam. Alkaline hydrolysis of a fat is termed **saponification**, because the alkali combines with the liberated aliphatic acid to form a soap.

Classification of Oils and Fats.



The so-called "essential" or volatile oils are not lipides, since they are not esters of aliphatic acids, but are derivatives of cyclic hydrocarbons. They are volatile in steam, and are not saponified by alkalies.

The chief *animal fats* employed industrially are lard, tallow, and butter.

Lard, obtained from the pig, is the mixed glyceride of stearic acid (40 per cent.), oleic acid (50 per cent.), linoleic acid (10 per cent.).

Tallow, or beef and mutton fat, contains mixed glycerides of stearic acid (33–50 per cent.), palmitic acid (10–20 per cent.), and oleic acid (50–60 per cent.).

Butter, or milk fat, occurs to the extent of 3–5.5 per cent. in mammalian milk, and is a complex mixture of the glycerides of oleic, lauric, myristic, palmitic, stearic, and arachidic acids. It is characterised by the presence of butyric acid, which makes up 4–6 per cent. of the total fatty acid present.

Human fat resembles tallow in many respects; its fatty acid content being largely determined by the fatty acids in the diet. At body temperature human fat is almost liquid, owing to its high percentage of glycerides of oleic acid.

Vegetable Fats.—The principal members of a large group are :—

Cacao butter (cocoa butter), from the beans of the cocoa tree (*Theobroma cacao*), is made of glycerides of stearic and oleic acids. It is used in pharmacy and in confectionery.

Cocoanut oil, from the fruit of the cocoanut tree (*Cocos nucifera*), is used largely in the preparation of edible fats, margarines, and “nut butter.”

Vegetable oils are divided into *drying oils*, which harden on exposure to light and air, and *non-drying oils*, which do not. Various intermediate forms are grouped as *semi-drying oils*. The property of “drying” depends on the presence of highly unsaturated acids in the glyceride.

Important non-drying oils are *rape seed oil* or *colza oil*, from the mustard; *almond oil*, *olive oil*, and *castor oil*, from seeds of *Ricinus communis*. Many of these are used as lubricants.

The commonest drying oils are *linseed oil*, from flax seed (*Linum usitatissimum*), *tung oil*, *hempseed oil*, *soya bean oil*, and *walnut oil*.

Marine oils include the *fish oils*, found almost uniformly distributed in the tissues of most fish; *liver oils*, chiefly from cod, shark, ling, halibut and skate, are of great therapeutic value as sources of the vitamins A and D; *blubber oils*, from the oleaginous tissues of the seal and the whale.

ANALYTICAL REACTIONS OF SIMPLE LIPIDES

Alkaline Hydrolysis (Saponification).—Shake up 5 ml. of olive oil or melted fat with 5 ml. of 20 per cent. sodium hydroxide. A white emulsion forms, consisting of a disperse phase of liquid fat in a continuous aqueous phase. Immerse the tube in boiling

water. The emulsion is unstable and resolves into a layer of oil on a layer of alkali. Hydrolysis takes place at the interface, and is aided by shaking the tube occasionally. After at least half an hour, remove and cool the tube. The contents show three layers: a lower one of alkali and glycerol, an upper one of unchanged oil, and an intermediate solid layer of soap, sodium oleate, which has been "salted-out" of the lower layer by the excess of alkali. Carefully pour off the liquid layers, and test for glycerol by means of the nitro-chromic test (p. 109). Rinse the small cake of soap with cold water to remove excess of alkali. Then add about 10 ml. of water, and boil till the soap dissolves.

Detection of Soap.—(a) Acidify about 3 ml. of solution with a few drops of HCl. The soap is decomposed and the liberated fatty acid separates out as a white precipitate.

(b) Add a few drops of 5 per cent. CaCl_2 to 3 ml. of soap solution. A white precipitate of calcium soap separates out. This reaction takes place in "hard" water when soap is added, and renders washing uneconomical.

(c) Add about 1 ml. of saturated NaCl to 3 ml. of soap solution. The soap is displaced, or "salted-out," and rises to the top of the liquid. This process is employed industrially in the purification of soap.

Detection of Glycerol.—Add a few drops of 5 per cent. potassium chromate and an excess of concentrated nitric acid to the mixture of oil and alkali after separation of the soap. A blue colour develops in the aqueous layer owing to the presence of glycerol liberated from the hydrolysed fat.

SOAPS

A soap is the metallic salt of a higher aliphatic acid, and is formed whenever fats are hydrolysed in an alkaline medium. Potassium, sodium, and ammonium soaps are dissolved readily by water to form colloidal solutions, and are used in washing. Calcium and magnesium soaps are insoluble, and represent a form in which these metals are excreted by the intestine.

Emulsification and saponification of the food fats is brought about by pancreatic lipase in the duodenum, preparatory to fat absorption.

WAXES

Waxes are formed by union of a higher alcohol and an aliphatic acid, and are non-glycerides. All are insoluble in water but soluble in fat solvents. They are much more resistant to hydrolysis than true fats, and are not attacked by the lipoclastic enzymes, hence, waxes are not utilisable in animal nutrition. When hydrolysed

they show their ester structure by giving rise to an aliphatic acid, and an alcohol.

The chief waxes are wool wax, beeswax, spermaceti, chinese wax, and carnauba wax.

Wool wax, or *lanolin*, comes from the fleece of sheep. It is a mixture of esters and free alcohols, including the sterols cholesterol and lanosterol.

Lanolin may absorb up to 80 per cent. of its weight of water, and is an important agent for the dermal administration of drugs. It is absorbed by the skin, and for this reason is hopefully described as a "skin food" by makers of cosmetics.

Beeswax, secreted by the honey-bee during digestion, is used for building the comb. It is chiefly myricyl palmitate, and is largely employed in the manufacture of polishes.

Spermaceti, obtained as a solid from the head oil of the sperm and other whales, is chiefly cetyl palmitate. Its principal industrial use is the manufacture of candles.

COMPLEX LIPIDES, OR LIPINES

Complex lipides resemble the fats physically, and yield aliphatic acids on hydrolysis. They differ chemically from the simple lipides in containing phosphoric acid or galactose in the molecule, usually associated with a basic nitrogen compound. The presence of nitrogen is indicated in the alternative name *Lipine*.

(1) **Phospholipides** are recognised and separated from other lipides by two chief properties :—

(i.) Insolubility in cold acetone. They may be precipitated from ethereal extracts of mixed lipides by addition of acetone.

(ii.) On hydrolysis they liberate phosphoric acid, the proportion being one molecule of acid for each molecule of lipine.

Four sub-groups of phospholipides are known :—

(a) **Lecithins**, glycerol esters of aliphatic acids and choline phosphate. They are universal in plants and animals, especially in embryonic tissue, nervous tissue, bone marrow, and egg-yolk, which may contain about 9 per cent.

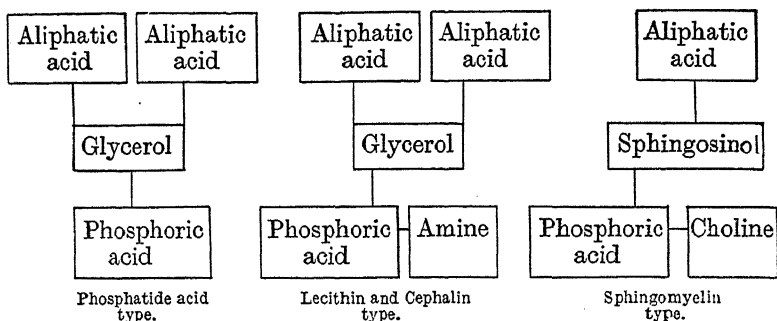
(b) **Cephalins**, glycerol esters of aliphatic acids and colamine phosphate. They are relatively abundant in brain and nervous tissue, and in egg-yolk. Cephalin, unlike any other phospholipide, has a powerful coagulative action on blood, and is believed by Howell to be identical with thrombokinese.

(c) **Sphingomyelins**, amides of an aliphatic acid with sphingosinol choline phosphate, are abundant in brain tissue, and closely

associated with the glycolipides, forming the mixture "protagon."

- (d) **Phosphatidic acids**, or phosphotidates, esters of aliphatic acids and glycerophosphoric acid. They are found in vegetable lipoids from seeds, fruit, and leaves.
- (e) **Bacterial phosphatides** separated from the waxy covering of the *Tubercle Bacillus*. On hydrolysis they yield: palmitic acid, oleic acid, a liquid fatty acid, now termed *phthioic acid*, glycerophosphoric acid, mannose, and inositol. These compounds differ from all the other phosphatide types.

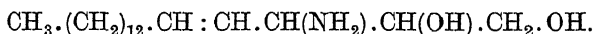
Structure of the Phospholipides.



Phosphatide Bases.—(1) *Colamine*, $\text{HO} \cdot \text{C}_2\text{H}_4 \cdot \text{NH}_2$ occurs in cephalins, and is a fairly strong base.

(2) *Choline*, $\text{HO} \cdot \text{C}_2\text{H}_4 \cdot \text{N}(\text{CH}_3)_3\text{OH}$, is found in lecithins. It and its derivative, acetyl choline, are of great physiological interest (p. 364).

(3) *Sphingosinol* is an 18-carbon amine containing two hydroxyl groups,



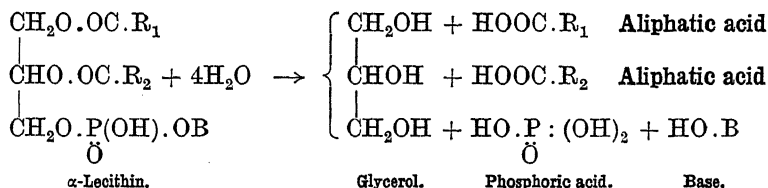
Lecithins are phospholipides containing choline as the only nitrogenous base. Each lecithin contains one molecule of glycerol, two of aliphatic acid, one of phosphoric acid and one of choline. These radicles are assembled in one of two ways. In the α -lecithins the phosphoric acid is joined to the terminal or α -carbon of the glycerol; in the β -lecithins, it is joined to the central or β -carbon atom. The aliphatic radicles differ in lecithins from different sources. Thus, egg-yolk lecithin contains stearic and oleic acid, liver lecithins contain palmitic or stearic acid along with oleic or arachidonic acid.

Lecithins are separated from the other phospholipides by precipitation from alcoholic solution on addition of cadmium chloride.

When pure, they are white, waxy solids, very hygroscopic and liable to oxidation.

They form emulsions spontaneously when mixed with water.

Hydrolysis of Lecithin.—Lecithin is attacked by lipase, which liberates the aliphatic acids, and by phosphatase, which liberates the phosphoric acid. Strong alkalis bring about complete hydrolysis in accordance with the equation :—



R_1 and R_2 are the acid radicles, *palmitic*, *stearic*, *oleic*, etc., as may be. $\text{HO} \cdot \text{B}$ is the basic radicle *choline*.

Cobra venom contains an esterase capable of liberating the unsaturated aliphatic acid from lecithin, and leaving a residual *lysolecithin*. Lysolecithins are powerful hæmolysins, and contribute by the fatal effect of injection of snake venom. They are antagonised by cholesterol, with which they form non-hæmolytic compounds.

Functions of Lecithin.—Lecithin is concentrated chiefly in egg-yolk, medullary sheaths of nerves and liver tissue. A 50-gm. egg contains about 1 gm. of lecithin in its 15-gm. yolk. The lipine forms a storage compound which is drawn on as the embryo develops.

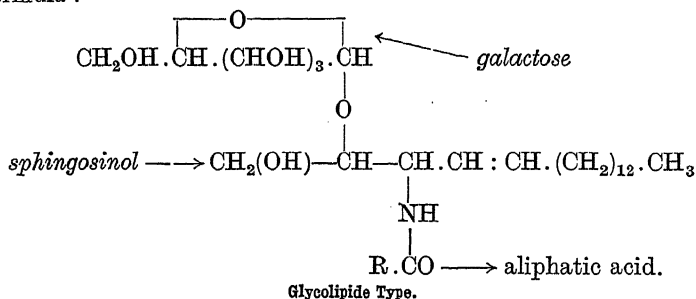
In nervous tissue it provides a reserve supply of choline, which in turn gives rise to the neurocrine, acetyl choline, necessary for the transmission of the nerve impulse.

(2) **Glycolipides** are of a glycoside structure, and on hydrolysis liberate : (a) galactose, a reducing sugar ; (b) sphingosinol ; and (c) an aliphatic acid. They occur chiefly in brain tissue, making up about 2–4 per cent. of fresh material, and are essential constituents of the medullary sheaths of nerves. Glycolipides, unlike the other lipides, are sparingly soluble in ether, and form part of the residue left from the ethereal extraction of brain substance. They are removed by means of warm alcohol, from which by fractional crystallisation they can be separated into : *phrenosin*, *kerasin*, *nervone*, and *hydroxynervone*.

Phrenosin, $\text{C}_{48}\text{H}_{81}\text{NO}_8$, is a white, micro-granular powder. On hydrolysis it yields : (a) galactose ; (b) sphingosinol ; and (c) phrenosinic acid.

Kerasin, *nervone*, and *hydroxynervone* resemble phrenosin but for the fact that each contains a different aliphatic acid united to the

sphingosinol galactoside. This is explained by means of the type formula :—



The nature of R, the aliphatic acid residue, depends on the glycolipide, as follows :—

Keratin yields lignoceric acid, $\text{C}_{23}\text{H}_{47} \cdot \text{COOH}$.

Phrenosin yields phrenosinic or α -hydroxylignoceric acid.

Nervone yields nervonic acid, $\text{C}_{23}\text{H}_{45} \cdot \text{COOH}$.

Hydroxynervone yields α -hydroxynervonic acid.

Glycolipides occur principally in the myelin sheaths of nerves. They were first isolated by Thudichum (1901).

Biological Significance of the Lipides.—A. Simple lipides

- (1) *Food Material*.—Fat in its various forms is one of the three great sources of carbon in the dietary. Containing less oxygen than the carbohydrates or the proteins, it is a highly concentrated form of fuel, 1 gm. liberating on combustion 9.3 kilocalories of energy.
- (2) *Food Reserve*.—On account of its insolubility in aqueous solutions, fat is stored readily in the organism, and is available to meet nutritional requirements. The adult man in health has a fat-content of about 120 gm. per kg. body weight ; more than half of this is reserve or storage fat.
- (3) *Heat Insulation*.—The subcutaneous lipides of the organism retard loss of heat from the surface of the body. They are specially concentrated in aquatic mammals, such as the whale, and in animals living in cold climates.
- (4) *Solvents*.—Dietary fat and storage fat carry in solution many important solutes, notably the fat-soluble vitamins A, D, and E. For this reason, perhaps, obese animals are less susceptible to acute dietetic deprivation of these particular vitamins.

B. Complex Lipides

- (5) *Structural Constituents*.—While storage fat is readily leached out of the dried tissues by extraction with ether or chloro-

form, some lipid material remains behind, and can only be removed completely by boiling alcohol. This is chiefly phosphatides, glycolipides, and sterols, and is believed to form part of the cell framework.

- (6) *Fat Transport*.—There is evidence that the phospholipide of the blood stream aids in the absorption and transportation of the aliphatic acids.
- (7) *Fat Metabolism*.—Fats are converted into phospholipides before undergoing desaturation and oxidative degradation.
- (8) *Storage Substrates*.—Choline and other reagents are held in an insoluble form in lipines until released by the appropriate enzymes.

GENERAL REFERENCES.

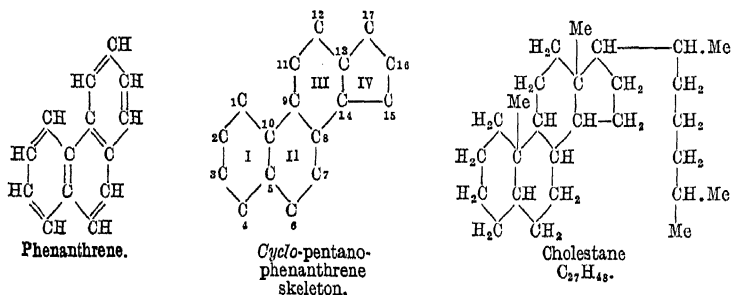
- BLOOR, W. R. (1925), "Biochemistry of the fats." *Chem. Rev.*, **2**, 243.
BULL, H. B. (1937), "Biochemistry of the Lipids." London.
FREYER, P. J., and F. E. WESTON (1918), "Technical Handbook of Oils, Fats and Waxes," 2nd Ed. Cambridge.
LEATHES, J. B., and H. S. RAPER (1925), "The Fats." Monographs on Biochemistry. London.
LEVENE, P. A. (1921), "Structure and significance of the phosphatides." *Physiol. Rev.*, **1**, 327.
MACCLEAN, H., and I. S. MACCLEAN (1927), "Lecithin and Allied Substances." London.
MITCHELL, C. A. (1918), "Edible Oils and Fats." London.
LEWKOWITSCH, J. (1921), "Chemical Technology and Analysis of Oils, Fats and Waxes." London.
SMITH, E. L. (1939), "Saponification in colloidal systems." *J. Chem. Ind.*, **58**, 87.
THIERFELDER, H., and E. KLENK (1930), "Der Chemie der Cerebroside und Phosphatide." Berlin.

CHAPTER XI

STEROIDS

THE lipide fraction obtained when tissues are extracted with fat-solvents usually contains, in addition to simple and complex fats, a varying amount of "lipoid" material that is not saponified by alkalis, and which consists chiefly of *sterols*, or solid alcohols derived from a saturated cyclic hydrocarbon, *cholestane*, $C_{27}H_{48}$. The first sterol to be recognised was obtained from gall stones, and was called "cholesterine" (Gk. *solid bile*), a name later changed to **cholesterol**, when an alcohol group was shown to be present in the compound. The generic term **sterol** was subsequently applied to substances resembling cholesterol. Eventually it was discovered that other important biological compounds, such as bile acids, hormones and vitamins, were closely related to cholesterol in that they contained the cyclic nucleus present in cholestane, but did not possess the long side-chain present in all sterols. These compounds are now termed **steroids**. Finally, the parent nucleus of all the sterols and steroids was identified as *cyclo-pentanophenanthrene*, a saturated hydrocarbon containing three 6-membered rings in phenanthrene configuration, and a 5-membered associated ring.

Definition.—Sterols and steroids are derivatives of a tetra-cyclic hydrocarbon, cyclo-pentanophenanthrene, $C_{17}H_{28}$, which in the sterols is methylated and carries a side-chain, thus constituting the cholestane system.



Classification.—Steroids differ so profoundly in their physiological activity that a chemical classification tends to obscure their practical

significance, and they are provisionally classified according to distribution and function.

Sterols.	Steroids.	
(1) Zoosterols	(4) Bile acids	(7) Toad poisons
(2) Phytosterols	(5) Saponins	(8) Vitamins
(3) Zymosterols	(6) Cardio-toxic glucosides	(9) Sex hormones
		(10) Carcinogens

STEROLS

Sterols occur universally in animals and plants, often dissolved in the storage lipides, or present in the form of esters in tissue fluids and organs. They are colourless, waxy solids, insoluble in water, and soluble in fat solvents. Chemically and biologically they are quite distinct from the simple and complex lipides, and their association with these compounds is due to their physical similarity. They are sub-divided into (i.) *zoosterols*, found in animals; (ii.) *phytosterols*, found in higher plants; and (iii.) *zymosterols*, or *mycosterols*, found in lower plants, such as fungi and moulds. They are all optically active compounds.

Natural Sterols

Sterol.	Formula.	M.p.	$[\alpha]_D$	Chief Sources.
<i>Zoosterols :</i>				
Cholesterol . . .	$C_{27}H_{45}.OH$	148°	—37°	All animal tissues
Coprostanol . . .	$C_{27}H_{47}.OH$	100°	+24°	Faecal lipoids
Spongosterol . . .	$C_{27}H_{47}.OH$	124°	—19.6°	Zoophytes
Ostreasterol . . .	$C_{29}H_{47}.OH$	143°	—44°	Molluscs
Agnosterol . . .	$C_{30}H_{47}.OH$	162°	+70°	Wool fat (lanolin)
Lanosterol . . .	$C_{30}H_{49}.OH$	140.5	+58°	" "
7-dehydrocholesterol	$C_{27}H_{43}.OH$			Liver Oils "
<i>Phytosterols :</i>				
Sitosterol . . .	$C_{29}H_{49}.OH$	138°	—24°	Higher plants, wheat germ
Stigmasterol . . .	$C_{29}H_{47}.OH$	170°	—45°	Soya bean, Calabar bean.
<i>Zymosterols :</i>				
Ergosterol . . .	$C_{28}H_{48}.OH$	161°	—180°	Yeast, ergot

Sitosterol occurs as a mixture of α -, β - and γ -isomers.

Cholesterol.—This sterol was isolated from biliary calculi in 1775, and has attracted continuous attention for over two hundred years; partly on account of its surgical importance as a constituent of gall stones, partly because of its unusual and complex chemical structure, and partly because it is a universally distributed zoosterol of obscure origin and significance.

Its ubiquity in the animal kingdom was demonstrated by Dorée,

in 1909. Cholesterol occurs normally free or combined (usually as an oleate or a palmitate) in tissues and secretions, notably brain, bile, blood and adipose deposits; it is found pathologically in biliary calculi, sebaceous cysts and atheromatous blood vessel walls.

Representative cholesterol values, expressed as percentage, are: human brain, fresh, 2.2; human brain, dried, 10.9; suprarenal gland, fresh, 5-7; sciatic nerve, dried, 5.6; spleen, kidney and lung, fresh, 0.3-0.5; animal fats, 0.1-0.35; human bile, 0.06-0.16; human milk, 0.03; blood plasma, 0.07-0.08; egg-yolk, fresh, 0.49; liver oils, 0.5-1.5.

In acute nephritis accompanied by oedema, the cholesterol content of the plasma increases in proportion to the oedema, and may reach a value of 390 mg. per 100 ml. Where there is no oedema, the cholesterol value may be unchanged. In chronic parenchymatous nephritis, the cholesterol level may rise as high as 730 mg. per 100 ml. plasma.

Cholesterol has not yet been found in any plant tissues, and, as Dorée has pointed out, its presence in a vegetable oil is evidence of adulteration with fat of animal origin.

Cholesterol is obtained readily by extracting powdered biliary calculi with boiling alcohol. On cooling, the sterol crystallises out in characteristic flat plates with one corner notched.

Origin of Cholesterol in Animals.—Preformed cholesterol in the mixed diet is believed to be sufficient to meet the sterol requirements of the adult animal, any excess being excreted by the intestine, either unchanged or reduced by bacterial action. Among herbivora, cholesterol does not occur in the diet, and must arise either by direct synthesis or by conversion of the vegetable phytosterols. Channon has shown that animals on a cholesterol-free diet are able to synthesise the sterol. Metabolic cholesterol is excreted in the bile, and enters the intestine, where it is changed by bacterial hydrogenation into the dihydro-derivative, coprostanol, formerly termed *coprosterol*, in which form it leaves the body.

Sitosterol and Stigmasterol are characteristic of higher plants, and are concentrated in tissues rich in lipides, especially the germ of the seeds. These sterols are restricted to the plant kingdom, and although they necessarily occur in the animal diet, there is no evidence either of their utilisation or conversion to zoosterols by higher animals, although it is possible that certain molluscs unable to manufacture cholesterol may obtain their characteristic *ostreasterol* from the isomeric phytosterols that occur in the algæ on which they feed.

Ergosterol, Provitamin D₂.—This sterol was isolated from the fungus *Ergot* by Tanret, but attracted little attention until 1927,

when it was shown to be converted into vitamin D₂ by solar or ultraviolet irradiation. It is found in many moulds, yeasts, higher plants, and in the liver oils and nervous tissues of animals. It crystallises readily, and can be separated from cholesterol and other sterols owing to its non-precipitability by digitonin, and its lower solubility in alcohol. Unlike cholesterol, it has three well-marked absorption bands in the ultra-violet region of the spectrum.

ANALYTICAL REACTIONS OF THE STEROLS

Cholesterol.—Cholesterol forms a characteristic precipitate with the alkaloid digitonin, by means of which it may be isolated and estimated. It also gives a great variety of colour reactions, notably :—

(1) *Acetic anhydride test* (Liebermann and Burchard)—About 5 drops of acetic anhydride are added to 1 ml. of a solution of cholesterol in chloroform. After mixing, concentrated sulphuric acid is added drop by drop until a violet colour appears. The violet soon changes into a stable emerald green, which has been employed for the colorimetric estimation of the sterol.

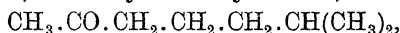
(2) *Trichloroacetic acid test* (Rosenheim, 1929)—Cholesterol and other natural sterols develop a red colour on being warmed with an excess of 90 per cent. trichloroacetic acid.

Ergosterol.—(1) *Chloral Hydrate Test* (Rosenheim, 1929).—When 1 mg. or less of ergosterol is added to about $\frac{1}{2}$ gm. of chloral hydrate melted in a water-bath, a carmine colour is produced which shows an absorption band at 500 m μ . This red colour soon changes into green, and then into a deep blue. A few natural sterols develop a red colour on warming with chloral hydrate, but the change to blue is seen only with ergosterol.

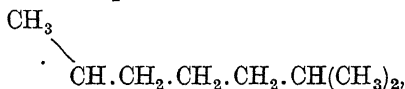
(2) *Bromine Test* (Tortelli-Jaffé).—A crystal of ergosterol is dissolved in 5 ml. of glacial acetic acid, and 1 ml. of a 2 per cent. solution of bromine in chloroform is added. A green ring appears at the surface of contact between the liquids.

Structure of the Sterols

(a) **The Side-chain.**—Cholesterol on oxidation yields an aliphatic ketone and a benzenoid residue. The former was identified by Windaus (1912) as methyl *iso*-hexyl ketone,



which indicates that the parent side-chain is



oxidation having taken place at the point of attachment of the $-\text{CH}$ group to the stable nucleus. By a similar procedure, the side-chains of the other sterols, and the steroid *cholic acid*, were separated and identified, and the following semi-structural formulæ were deduced:—

Cholesterol $R.\text{CH}(\text{CH}_3).\text{CH}_2.\text{CH}_2.\text{CH}_2.\text{CH}(\text{CH}_3)_2.$

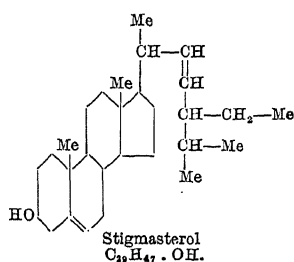
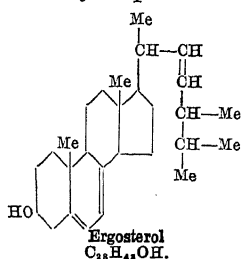
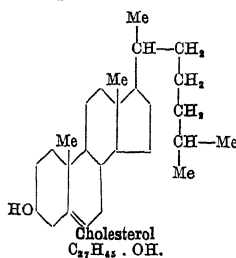
Stigmasterol $R.\text{CH}(\text{CH}_3).\text{CH}=\text{CH}.\text{CH}(\text{C}_2\text{H}_5).\text{CH}(\text{CH}_3)_2.$

Ergosterol $R.\text{CH}(\text{CH}_3).\text{CH}=\text{CH}.\text{CH}(\text{CH}_3).\text{CH}(\text{CH}_3)_2.$

Cholic acid $R.\text{CH}(\text{CH}_3).\text{CH}_2.\text{CH}_2.\text{COOH}.$

(b) **The Nucleus.**—Subtracting the value of the side-chain, C_8H_{17} , from the formula for cholesterol, the value $\text{C}_{19}\text{H}_{28}.\text{OH}$ is obtained for the nucleus. The stability of the sterol, and the ratio of C to H in the nucleus suggest the presence of four hydrogenated rings fused together. One of these rings carries the hydroxyl group, and on oxidation is broken at this point, giving rise to a monocarboxy acid. By chemical methods this acid can be degraded until it has lost two $-\text{CH}_2$ groups, showing that it has the side-chain, $-\text{CH}_2.\text{CH}_2.\text{COOH}$, attached to a second ring (II). Since this side-chain was formed by the opening up of another ring at its weakest point, the position of the hydroxyl group in the original ring (I) can be located. *Lithocholic acid* contains a similarly placed hydroxyl group, and its nucleus can be oxidised and degraded in the same way. Cholic acid, however, contains three hydroxyl groups in its nucleus, and the oxidation derivatives indicate that each of them is attached to a different ring, which suggests the presence of a phenanthrene system of three benzenoid rings. Meanwhile, the preparation of cholestane and other reduction derivatives from various steroids supported the belief that the nucleus of all these compounds was similar in pattern, although the existence of various isomeric forms aggravated the difficulties of the research.

It was not found possible to assign a benzenoid structure to the remaining ring (IV). In 1932, Rosenheim and King suggested that rings I, II and III occurred as a phenanthrene system, and, subsequently, Wieland completed the formula by showing that ring IV was derived from cyclo-pentane.



Coprostanol (coprosterol) is the reduction derivative of cholesterol obtained by hydrogenation of the double bond in ring II.

Ergosterol (provitamin D₂) differs from cholesterol by an additional methyl group and two double bonds, one of which is in the side-chain. Stigmasterol differs from cholesterol only in the side-chain, which has a double bond and an additional ethyl group.

STEROIDS

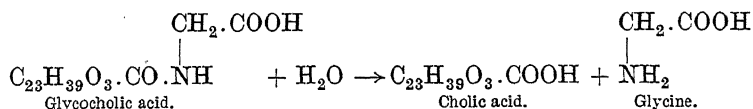
The Bile Acids.—The bitterness of gall was proverbial when Israel dwelt in Egypt, and is a characteristic property of the bile of all animals. It is due to the presence of two acids, *glycocholic acid* and *taurocholic acid*, which are (manufactured) in the liver. On hydrolysis, these give rise to a parent bile acid, **cholic acid**, which is often accompanied by related acids. Methods of estimation are not yet entirely satisfactory, but it is believed that human fistula bile contains 0.5 to 1.0 per cent. of the bile acids, present as esters, or "bile salts."

Natural Bile Acids

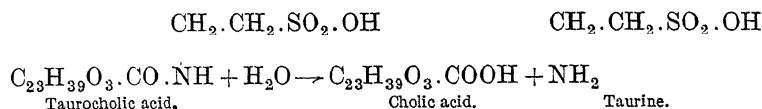
Acid.	Formula.	Source.
Cholic acid	$C_{23}H_{39}O_3 \cdot COOH$	Mammalian and non-mammalian bile
Deoxycholic acid	$C_{23}H_{39}O_2 \cdot COOH$	" "
Hydro-deoxycholic acid	$C_{23}H_{39}O_3 \cdot COOH$	" "
Lithocholic acid	$C_{23}H_{39}O \cdot COOH$	" "
Scymnol	$C_{26}H_{45}O_3 \cdot COOH$	Shark bile

The Bile Esters ("Bile Salts")

Glycocholic acid is derived from glycine and cholic acid, and is resolved into them on acid hydrolysis.

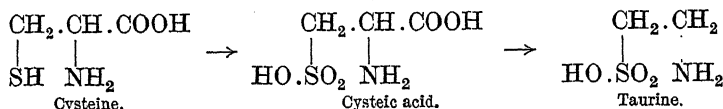


Taurocholic acid is a derivative of taurine and cholic acid :



Taurine, amino-ethylsulphonic acid, is derived from the amino

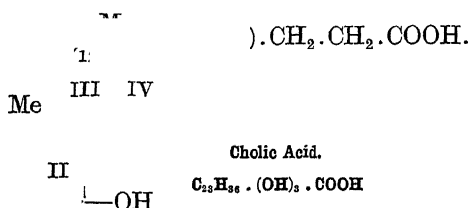
acid cysteine by oxidation to cysteic acid, followed by decarboxylation :



Structure of the Bile Acids

Bile acids are classified in reference to a reduction product, **cholanic acid**, which is common to all three. Cholanic acid has the same nucleus as cholestane, but the side-chain has been shortened to $-\text{CH}(\text{CH}_3) \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$.

Cholic acid is a trihydroxy cholanic acid in which the hydroxyl groups occupy positions 3, 7 and 12 in the nucleus.



There is evidence that cholic acid from pig bile has its second hydroxyl group at position 6 instead of 7.

The acids which accompany cholic acid differ in their degree of reduction. Thus, *deoxycholic acid* has the OH at 7 replaced by H; *hydro-deoxycholic acid*, from pig bile, has the OH at 12 replaced by H; while *lithocholic acid* has the $-\text{OH}$ group at 7 and at 12 replaced by H.

Scymnol, $\text{C}_{27}\text{H}_{46}\text{O}_5$, occurs as a sulphuric ester in shark bile, and is of interest in that it represents a compound intermediate in type between cholesterol and cholic acid, and may indicate their biochemical relationship. Scymnol has the same nucleus as cholic acid, but carries a different side-chain,



Preparation of Bile Esters.—Mix a paste made of 50 ml. of ox bile and 10 gm. of animal charcoal, and evaporate to dryness on a water-bath, stirring at intervals. Powder the residue, and boil with 100 ml. of 95 per cent. alcohol for about half an hour. Cool, and filter into a dry dish. Add ether until the mixture begins to form a permanent cloud. Cover, and leave overnight at low temperature. A crystalline mass of bile esters separates out, and may be filtered off, and purified by reprecipitation from alcohol.

The esters are separated by fractional crystallisation from water, in which taurocholic acid is much more soluble than glycocholic acid. Taurocholic acid is very bitter; glycocholic acid has a characteristic bitter-sweet taste.

The significance and analytical reactions of the bile acids and esters are discussed subsequently in Chapter XV.

Steroid Glucosides

The Neutral Saponins.—Soapwort (*Saponaria*) and many other plants contain a class of glucoside characterised by the property of yielding soap-like froths in aqueous solution, and capable of acting as emulsifiers and detergents, and hæmolyzing blood cells, even in very low concentration.

On hydrolysis, saponins yield glucose and an aglucone (p. 78) termed a *genin*. Thus, the common foxglove (*Digitalis purpurea*) contains a group of saponins, including *digitonin*, which yields *digitogenin*, $C_{27}H_{44}O_5$.

The Cardio-toxic Glucosides.—Saponins are often associated with glucosides having a powerful action on the heart, especially in plants belonging to the order *Apocyanaceæ*.

The most familiar of the aglucones obtained from these glucosides belong to the digitalis group, used for over a hundred years in medicine, and the strophanthus group, which has a longer history as a source of arrow poisons. Representative members are *digitoxigenin*, $C_{23}H_{34}O_4$, and *strophanthidin*, $C_{23}H_{32}O_6$, but at least nine have been isolated and identified.

Both the saponin genins and the cardio-toxic aglucones possess the cyclo-pentanophenanthrene nucleus characteristic of the sterols, to which is affixed a side-chain which determines the specific properties of the steroid. Thus, cardio-toxicity is associated with the presence of an unsaturated lactone ring attached to ring IV of the nucleus.

The Toad Poisons.—The “ugly and venomous” toad owes its reputation to the presence of *bufotoxin* in the secretion of its skin glands. Related toxins have been obtained from other species of the animal.

These toad poisons in their physiological action resemble the cardio-toxic glucosides, and one of them has long been used in China as the drug Ch'an Su. Bufotoxin on hydrolysis yields arginine, suberic acid, and a toxic steroid, *bufotalin*, $C_{26}H_{36}O_6$.

Steroid Vitamins

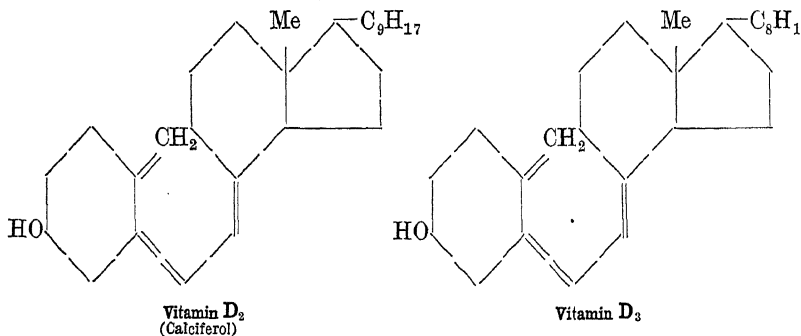
In 1927, it was shown by Rosenheim, Pohl and Windaus that ultra-violet irradiation of pure ergosterol produced a series of

isomeric steroids ($C_{28}H_{44}O$), one member of which was highly anti-rachitic and had the calcium-controlling, or *calcio-kinetic*, properties of the natural vitamin D obtained from liver oils.

Ergosterol \rightarrow lumisterol \rightarrow tachysterol \rightarrow calciferol \rightarrow toxisterol.

Calciferol is the anti-rachitic steroid obtained artificially. It was first regarded as being identical with vitamin D, and ergosterol was accepted as being the natural provitamin. However, in 1934, Waddell found that both irradiated cholesterol and cod-liver oil concentrates were more effective than calciferol, when given in equivalent dosage to rachitic chickens, and now it is believed that several different vitamins of the D type exist, including D, the anti-rachitic factor in natural liver oils, D₂, or calciferol, and D₃, the factor obtained from 7-dehydrocholesterol, which is its provitamin in crude cholesterol.

Structure of the D Vitamins.—These steroids are characterised by the presence of a nucleus in which ring II has been opened up to form an unsaturated chain. This occurs when provitamins are “activated” by irradiation or chemical treatment,



The side-chain, C_9H_{17} of D₂ is the same as that of ergosterol; and the side-chain, C_8H_{17} of D₃ is the same as that of cholesterol.

The Sex Hormones

Specific compounds elaborated in the gonad tissue of vertebrates are necessary for the mature development and functional maintenance of the genetically determined sexual type. These compounds are classed as *gynæcogens*, or female hormones, and *androgens*, or male hormones. Gynæcogens are divided into (i.) the œstrone group of follicular hormones, and (ii.) the hormone of the corpus luteum. Androgens are represented by the androsterone group of hormones. All these compounds are steroid in character, and

they or their precursors have been detected in a variety of animal and plant materials. For example, *œstrone*, the follicular hormone is manufactured chiefly in the ovarian follicle, and is a constant constituent of the urine during pregnancy, but it has been isolated also from male urine, and from the oil of the palm tree nut. In 1930, Marrian showed that a variety of hormones closely related to *œstrone* could be separated from both male and female urine, and it is now recognised that both gynæcogens and androgens are present in both sexes.

As Elderfield has observed, the classification of these factors as male hormones and female hormones is misleading. The biological effects of a particular hormone are not restricted to the reproductive organs of one sex, and the same compound may display the dual action of both male and female hormones. Provisionally, a male hormone is described as having the essential property of promoting growth of the secondary sexual structures in the male, such as the comb and plumage of capons and the seminal vesicles of castrated male rats. A female hormone has the property of promoting the œstrus cycle and uterine enlargement.

The work of Dodds and his colleagues has shown that female hormones need not necessarily be steroid in character. A stilbene derivative has been obtained, diethyl stilbœstrol, that has nearly three times the potency of the natural hormone, *œstrone* (p. 433).

Female Sex Hormones

The Follicular Hormone.—This hormone occurs in the urine of pregnancy in four forms: (1) *œstrone*, $C_{18}H_{22}O_2$, (2) *œstriol*, $C_{18}H_{24}O_3$, (3) *equilin*, $C_{18}H_{20}O_2$, and (4) *equilenin*, $C_{18}H_{18}O_2$; and also occurs in the ovary as (5) *œstradiol*, $C_{18}H_{24}O_2$. All these compounds are 3-hydroxy-13-methyl-derivatives of the *cyclopentanophenanthrene* nucleus, and carry a ketone group or a hydroxyl group in position 17. They are excreted in the urine chiefly as esters of glycuronic acid, in which form they are of low activity. These compounds are representing **Estrogens**.

The Corpus Luteum Hormone.—Only one form is known to exist, *progesterone* (progestin, luteosterone), $C_{21}H_{30}O_2$, which has been obtained from the corpus luteum and from urine of pregnancy, where it is accompanied by an inactive precursor (or reduction derivative) the alcohol, *pregnandiol*, $C_{21}H_{33}O_2$. By degradation of the side-chain in stigmasterol, Butenandt has obtained progesterone, thus proving the steroid character of the hormone.

The structural formulæ of the sex hormones is given in Chapter XXIV.

Androgens

The Testicular Hormones.—From male urine, (1) *androsterone*, and (2) *dehydro-androsterone*, have been isolated; *testosterone* (3) has been obtained from testicular extracts, and has been prepared, also, from cholesterol. *Androstadiol* (4) has been obtained by reduction of androsterone; it is the diol corresponding to cestradiol, and is about three times as potent as the parent androsterone, but, unlike cestradiol, it has not yet been isolated from natural sources. The androgens resemble the gynæcogens in general structure, but differ in their degree of saturation. Testosterone is the most powerful. Chemically, the androgens are simple keto or hydroxy derivatives of a parent steroid, *androstane* (p. 436). The suffix *diol* denotes the presence of two hydroxyl groups.

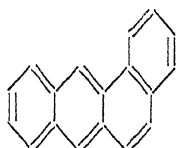
The Adrenal Cortex Hormones

The adrenal cortex is rich in sterols, and its internal secretion appears to be steroid in character. The cortin complex, originally obtained in crystalline form by Kendall, Reichstein and their colleagues, has now been resolved into a mixture of active and inactive compounds, some of which contain the C_{19} nucleus. The term *corticosterone* is applied to the compound, $C_{21}.H_{30}O_4$, which is reported to have the biological action of the cortical hormone. A steroid with weak androgenic properties has also been isolated from the lipid fraction of the cortex (p. 415).

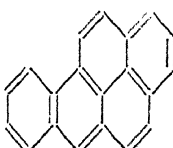
Carcinogenic Hydrocarbons

In 1915, Yamagiwa showed that application of tar was capable of evoking malignant changes in the skin of rodents. The production of these skin cancers was verified by many workers, although it was realised that animals differed in their susceptibility and tars differed in their carcinogenic property. In 1924, Kennaway obtained and isolated active carcinogens from the mixture of tars produced when pure acetylene or pure isoprene is heated with hydrogen, thus showing that the agent was a hydrocarbon, and not a contaminant. The synthetic and natural carcinogenic tars resembled one another in the possession of an intense fluorescent spectrum, which suggested the presence of polycyclic nuclei.

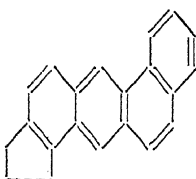
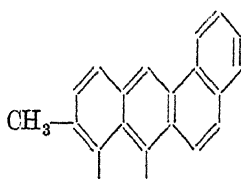
A number of such compounds were prepared and examined, and at least four were found to possess unmistakable carcinogenic properties. They are: (1) 1:2-benzanthrene, (2) 1:2-benzpyrene, (3) 5:6-*cyclo*-pentano-1:2-benzanthrene, and (4) methyl colanthrene, which was obtained from deoxycholic acid, and is very active.



1:2-Benzanthrene.



1:2-Benzpyrene.

5:6-cyclo-Pentano-
1:2-benzanthrene.

Methyl colanthrene.

Now, although none of these compounds is a true steroid, they all contain polycyclic nuclei, and, furthermore, the fact that methyl colanthrene has been prepared from a naturally occurring bile acid suggests that they must be considered as possible types of compounds produced in abnormal sterol metabolism.

9:10-dimethyl benzanthrene is a powerful carcinogen, and its local application may induce a skin cancer within thirty-five days.

Two at least of the synthetic carcinogens, namely, 1:2-benzpyrene and 5:6-cyclo-pentano-1:2-benzanthrene are also active oestrogens.

GENERAL REFERENCES

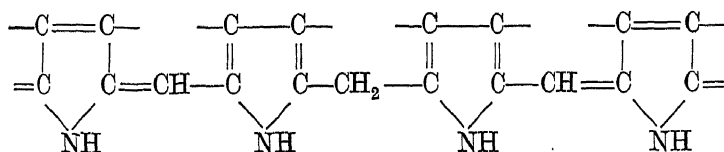
- BARRY, G., and J. W. COOK, *et al.* (1935), "Pure hydrocarbon carcinogens." *Proc. Roy. Soc., B*, **117**, 318.
- BILLS, C. E. (1935), "Physiology of the sterols." *Physiol. Rev.*, **15**, 1.
- DODDS, E. C. (1935), "Structure of certain sex hormones, vitamins and carcinogenic agents in relation to their biological activity." *Ergeb. Physiol.*, **37**, 264.
- DORÉE, C. (1937), "Recent history of the sterol group." *School Sci. Rev.*, **18**, 337.
- FIESER, L. F. (1937), "Chemistry of Natural Products Related to Phenanthrene." 2nd Ed. New York.
- FRIEDMANN, E. (1937), "Sterols and Related Compounds." Cambridge.
- SCHOENHEIMER, R., and E. A. EVANS (1937), "Chemistry of the steroids." *Ann. Rev. Biochem.*, **6**, 139.
- STRAIN, W. H. (1938), "Sterols, bile acids, and related compounds." *Organic Chemistry*, Ed. Gilman, **2**, 1220.

CHAPTER XII

BIOLOGICAL PIGMENTS : PYRROLE DERIVATIVES, CAROTINOIDS, FLAVINS

A. PYRROLE DERIVATIVES

THE red world of the animal and the green world of the plant owe their colour to the presence of pigments derived from a simple heterocyclic compound, pyrrole, $C_4H_4.NH$, which is arranged as tetrapyrrole groups of four units. Two great classes of these pigments exist: the *linear tetrapyrroles*, represented by the bile pigments; and the *cyclic tetrapyrroles*, or porphyrins, found in the blood pigments and in chlorophyll.



Tetrapyrrole skeleton.

Natural Linear Tetrapyrroles

Name.	Formula.	Source.
Bilirubin	$C_{33}H_{36}O_6N_4$	Bile
Biliverdin (Dehydrobilirubin)	$C_{33}H_{34}O_6N_4$	Bile
Mesobilinogen	$C_{33}H_{44}O_6N_4$	Reduced bilirubin
Urobilinogen	$C_{33}H_{48}O_6N_4$	Urine
Mesobilin	$C_{33}H_{42}O_6N_4$	Urine
Urobilin (stercobilin)	$C_{33}H_{46}O_6N_4$	Urine, Intestinal contents
Uteroverdin	$C_{33}H_{34}O_6N_4$	Dog placenta
Bilipurpurin (phylloerythrin)	$C_{34}H_{36}O_6N_4$	Bile

The hepatic secretion of man and other animals is deeply coloured owing to the presence of pigments, chiefly *bilirubin* and *biliverdin*. Bilirubin is the chief pigment in human bile, to which it imparts a golden-yellow colour. Biliverdin, an oxidation derivative of bilirubin, is found in herbivora and other animals, and imparts an emerald green colour to the bile. When both pigments are present,

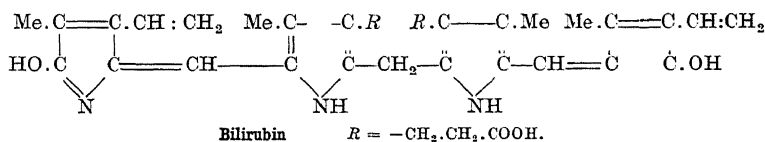
as in ox bile, the colour may vary from brown to green. These pigments are waste products derived from hæmoglobin.

Bilirubin, $C_{33}H_{36}O_6N_4$, occurs in bile as a soluble sodium bilirubinate; in biliary calculi it is present as an insoluble calcium salt. It can be prepared from evaporated bile residues or powdered gallstones by the following method of successive extraction: (i.) ether, (ii.) hot water, (iii.) 10 per cent. acetic acid, (iv) alcohol, and (v.) hot glacial acetic acid. By this process the following substances are removed: sterols, bile salts, biliverdin, and inorganic salts. The residue is dried and extracted with hot chloroform, which removes the bilirubin. On cooling, bilirubin crystallises out.

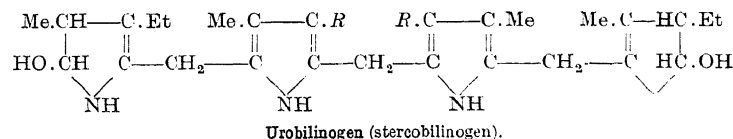
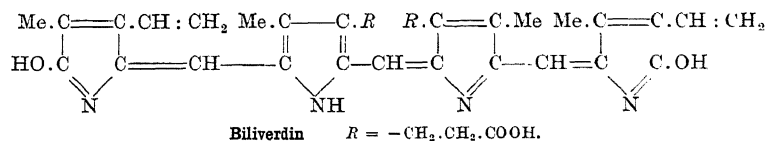
Bilirubin is a red-brown tetrapyrrole, insoluble in water, dilute acids, and the common fat-solvents. It is soluble in hot chloroform and in alkalis, in which it dissolves to form salts. The solutions show no characteristic absorption spectrum.

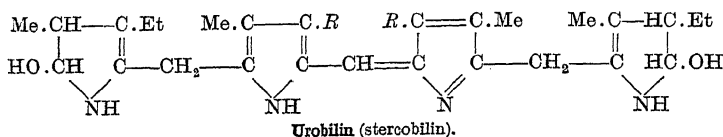
Structure.—Mild oxidation converts bilirubin into the green pigment, *biliverdin*; more powerful oxidants resolve it into four pyrrole units. Bilirubin does not combine with metals to form porphyrans, thus indicating that the formula is an open chain of pyrrole units, not a closed tetrapyrrole or porphyrin.

When bilirubin is reduced by sodium amalgam it is converted to a colourless derivative, mesobilinogen, which is reoxidised by atmospheric oxygen to a yellow compound, *mesobilin*, that resembles *urobilin* (stercobilin), the waste pigment present in urine and intestinal contents.



By reduction of the two vinyl groups $-\text{CH:CH}_2$, to ethyl groups, Et, $-\text{CH}_2.\text{CH}_3$, mesobilin is formed from bilirubin.





Biliverdin, or dehydrobilirubin, is derived from bilirubin by loss of two hydrogen atoms in the region of the central linkage of the tetrapyrrole chain. It is formed spontaneously when bilirubin is oxidised by exposure to air in alkaline solution, or treated with hydrogen peroxide. The pigment is precipitated by acidification, and any unchanged bilirubin removed by chloroform extraction. Biliverdin is a dark green amorphous solid, insoluble in water, ether or chloroform. It dissolves in alkalis to form salts, and is freely soluble in glacial acetic acid. These solutions have a bright green colour, but no characteristic visible spectrum. Biliverdin is the precursor of bilirubin in the formation of bile pigment from hæmoglobin.

Urobilin, or stercobilin, is a brown pigment derived from bilirubin by bacterial reduction in the intestine. It is reabsorbed into the blood, and reduced still further to the colourless chromogen, *urobilinogen*, in which form it is excreted in the urine. Urobilin is precipitated from urine by saturation with ammonium sulphate and extraction with alcohol. In solution, it shows a distinct absorption band in the region $b - F$ ($486 \text{ m}\mu - 508 \text{ m}\mu$). Addition of zinc chloride or acetate to the neutral solution causes a green fluorescence and the appearance of an additional absorption band near the b line. Urobilin is the chief derivative of the bile pigments that occurs in urine, and may also be accompanied by mesobilin, which formerly was regarded as being identical with urobilin.

Urobilinogen, the colourless precursor of urobilin, is one of the normal constituents of fresh urine, and may be greatly increased in amount by conditions of intestinal stasis and increased intestinal putrefaction. When urine is exposed to air, or treated with mild oxidising agents, urobilinogen is converted to urobilin, and the colour of the urine darkens. Like urobilin, it is precipitated by saturation with ammonium sulphate, but, unlike urobilin, it is soluble in ether, and thus may be extracted. When pure, urobilinogen is a colourless crystalline solid. In solution it shows no absorption bands and no fluorescence on addition of zinc salts.

Urobilinogen, unlike urobilin, gives an immediate red colour with Ehrlich's aldehyde reagent in acid solution (p. 149).

Urobilin and urobilinogen do not usually occur free in urine, but in some loosely combined forms. It is possible that *urochrome*, the characteristic yellow pigment of urine, is a urobilin derivative.

Free urobilin and urobilinogen are excreted in febrile and other conditions accompanied by rapid loss of protein, and in the recovery stage of obstructive jaundice.

Natural Cyclic Tetrapyrroles, or Porphyrins

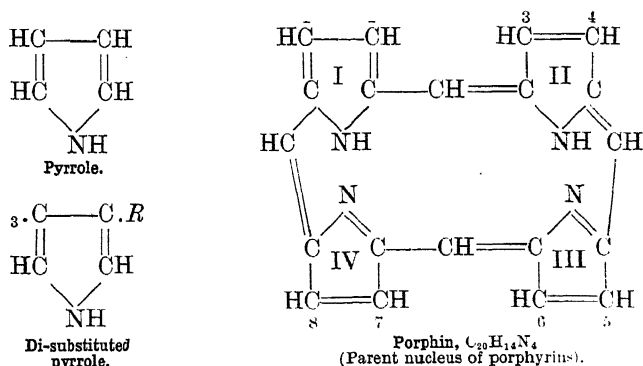
Porphyrins are widely distributed pigments of high stability. The nitrogen in the tetrapyrrole cluster readily combines with metals, such as Fe, Cu, Mg, and Mn, to form metallo-porphyrins, or *porphyrans*, having catalytic properties.

Porphyrin proteins, such as hæmoglobin, participate in oxygen transport; others, such as cytochrome, are concerned in the oxidation mechanisms of all living tissues.

Name.	Formula.	Source.
Protoporphyrin . . .	$C_{34}H_{34}O_4N_4$	Hæmoglobin
Hæmatoporphyrin . . .	$C_{34}H_{38}O_6N_4$	Hæmoglobin
Ætioporphyrin I. . .	$C_{32}H_{38}N_4$	Chlorophyll
Ætioporphyrin III. . .	$C_{32}H_{38}N_4$	Hæmoglobin
Coproporphyrin . . .	$C_{36}H_{38}O_8N_4$	Urine, yeast
Uroporphyrin . . .	$C_{38}H_{38}O_{16}N_4$	Urine, Turacou feathers

General Structure of the Porphyrins

Knowledge of the porphyrins has been reached by the convergence of work on chlorophylls, chiefly by the Willstätter school, and on the blood pigments, by Hans Fischer; and important contributions have been made by Stoll, Conant, Marchlewski, Kuhn, and Küster. The properties of the porphyrins as a class suggests a ring structure composed of four stable units. On oxidation of a porphyrin, these units are obtained in the form of di-substituted pyrroles, one substituent of which is always a methyl group.



The hæmochromes include the iron-containing chromoproteins, hæmoglobin, erythrocrucorin, chlorocrucorin and hæmoerythrin, and the copper-containing chromoprotein, hæmocyanin. The cytochromes are three in number, and are found in all aerobic cells.

THE HÆMOCHROMES, OR BLOOD PIGMENTS

	Hæmoglobin	Erythrocrucorin	Chlorocrucorin	Hæmocyanin	Hæmoerythrin
<i>Distribution</i> .	Vertebrates	Many invertebrates	Polychate worms	Molluscs Arthropods	Gephyreans
<i>Colour</i> .	Red	Red	Green	Blue	Red
<i>Prosthetic group</i>	Hæm	Hæm	Hæm	Thio-peptide	
<i>Metal</i> .	Fe	Fe	Fe	Cu	Fe
<i>Location</i> .	Corpuscles, Muscle	Corpuscles	Plasma	Plasma	Corpuscles or plasma
<i>M. Wt.</i> .	68,000	17,000 to 5×10^4	300,000 to 5×10^4	300,000 to 5×10^4	17,000 to 68,000

Hæmoglobin, the dominant pigment of animal life, is found in the blood of all vertebrates. It is a chromoprotein, made up of 94 per cent. of globin and 4–5 per cent. of the porphyrin, **hæmhæm**, the residue being mostly lipid material. Not only does the hæmoglobin differ in different species but there is evidence for the presence of more than one form of hæmoglobin in the same animal, and obscure racial relationships may be traced this way. These species-differences are ascribed to differences in the globin component of the molecule. Crystalline hæmoglobin can be obtained directly from the blood of horses, dogs, and rats, after “laking,” or hæmolysis by addition of water or ether.

Derivatives of hæmoglobin.

A. *Addition compounds*: oxyhæmoglobin, methæmoglobin, carboxyhæmoglobin, sulphæmoglobin.

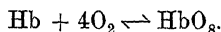
B. *Decomposition products*: hæmochromogen, hæmin, hæm, hæmatoporphyrin.

Oxyhæmoglobin, HbO_2 , a scarlet compound of hæmoglobin containing two atoms of displaceable oxygen for each atom of iron in the molecule. It forms spontaneously when a solution of hæmoglobin is shaken with air, and it is responsible for the transport of oxygen in the blood stream.

In concentrations of 1:1,000 to 1:10,000 oxyhæmoglobin has a characteristic spectrum with two absorption bands between the D and the E lines. The α -band is the narrower and more distinct, and lies on the D line, the middle of the band being at $579\text{m}\mu$. The middle of the β -band is about $542\text{m}\mu$.

Oxyhæmoglobin has a third band (Soret's band), located in the ultra-violet region of the spectrum between G and H, its centre being at 415 $m\mu$. When oxyhæmoglobin is reduced, this band is displaced towards the visible spectrum. On exposure to low atmospheric or oxygen pressure, or when treated with reducing agents such as hydroxylamine, sodium hydrosulphite, ammonium sulphide, oxyhæmoglobin is readily reconverted into hæmoglobin, which shows a single diffuse band between the D and E lines.

The Interaction between Hæmoglobin and Oxygen.—Crystallised hæmoglobin, irrespective of its source, contains 0.0335 per cent. of iron, which is in the ferrous form. One gram of hæmoglobin combines with 1.34 ml. of oxygen, at 0° C. and 760 mm. Hg (atmospheric pressure), a ratio corresponding to two atoms of O for each atom of Fe. The union is molecular; oxygen unites and dissociates in the form of O_2 , without oxidising the Fe. Assuming that a molecule of Hb contains only one atom of Fe, the molecular weight is approximately 16,700. But direct determinations of sedimentation rate and osmotic pressure indicate a value of about 68,000, which is four times as large. Unless this is due to molecular aggregation in solution, the formula for oxyhæmoglobin is HbO_8 , and the reversible equation is :



Methæmoglobin, HbO or $HbOH$, is formed when hæmoglobin is oxidised in alkaline solution with permanganate or peroxide, or when potassium ferricyanide is added to oxyhæmoglobin. The colour of the solution turns chocolate-brown, and the characteristic spectrum of methæmoglobin develops. In acid solution there is an absorption band towards the red end of the spectrum, between C and D, its centre being about 634 $m\mu$. In alkaline solution, two absorption bands are seen, resembling those of oxyhæmoglobin but differing in that the β -band is sharper than the α -band. Methæmoglobin is formed also by the action of nitrobenzene, pyrogallol, acetanilide, and other phenols and amines, and may appear in the urine accompanying the hæmaturia due to poisoning by chlorate, nitrate, or phenols.

In methæmoglobin, the iron has been oxidised to the ferric form, and the pigment is no longer able to take part in oxygen transport as it does not yield up its oxygen on exposure to low pressures. By the action of reducing agents, or by intravenous injections of glucose into the circulation, methæmoglobin is converted into hæmoglobin, and thus restored to physiological utility.

Carboxyhæmoglobin, or carbon monoxide hæmoglobin, $HbCO$, is formed by the action of CO on hæmoglobin or oxyhæmoglobin,

from which it displaces the oxygen. The spectrum shows two absorption bands resembling those of oxyhæmoglobin but shifted more towards the violet end; the centre of the α -band being at $570\text{ m}\mu$, and the β -band at $535\text{ m}\mu$.

Distinction between oxy- and carboxyhæmoglobin :—

(1) Carboxyhæmoglobin is more pink or "cherry red" than oxyhæmoglobin solutions of the same concentration, and on dilution the colour of the carboxy compound remains pinkish, while the oxyhæmoglobin turns yellowish.

(2) On treatment with ammonium sulphide or similar reducing agents, carboxyhæmoglobin is unchanged, while oxyhæmoglobin is converted to hæmoglobin. This test can best be followed by means of a spectroscope. By the action of a strong reducing agent, such as sodium hydrosulphite, aided by heat, carboxyhæmoglobin can be converted into hæmoglobin.

(3) A few drops of dilute copper sulphate produce a greenish-brown precipitate when added to solutions of oxyhæmoglobin, and a crimson precipitate when added to carboxyhæmoglobin.

(4) By means of the reversion spectroscope, carboxyhæmoglobin can be detected and estimated in presence of oxyhæmoglobin.

DECOMPOSITION PRODUCTS OF HÆMOGLOBIN

Hæmochromogen is a chromoprotein formed by the action of alkalis and reducing agents on hæmoglobin. When a dilute solution of blood is warmed with an alkali the colour changes from red to greenish-brown. This is due to (1) formation of methæmoglobin, and (2) its decomposition into the protein *globin* and the iron porphyrin *hæmatin* or *methæm*. At the same time, the alkali denatures the liberated protein. If now a reducing agent be added, such as sodium hydrosulphite, the hæmatin is converted to reduced hæmatin or hæm. Hæm rapidly recombines with the denatured globin to form a new chromoprotein *hæmochromogen*.

In alkaline solution this hæmochromogen has a bright carmine colour, and shows two bands somewhat like those of oxyhæmoglobin but nearer the violet end of the spectrum. The α -band is the darker and narrower, and its centre is at $556\text{ m}\mu$, almost midway between the D and the E lines. The β -band covers the E and the b lines, and has its centre about $528\text{ m}\mu$. Since the spectrum of hæmochromogen can be detected in dilutions at which the spectra of oxy- and carboxyhæmoglobin are invisible, its formation is sometimes used as a test for traces of blood.

The pigment can be distinguished rapidly from oxyhæmoglobin by its stability to reducing agents.

Hæmatin, or **methæm** or oxidised hæm, $\text{C}_{34}\text{H}_{32}\text{N}_4\text{O}_4\text{FeOH}$, a base which in reduced form occurs united to protein in hæmo-

chromogens. It is a dark, amorphous powder, insoluble in water and many organic solvents, but dissolves in alkalis or in glacial acetic acid forming solutions termed *alkaline* and *acid hæmatin*, respectively. The change of colour blood undergoes when warmed with alkalis or acids is due to the formation of the corresponding hæmatin.

When a mixture of alcohol and ether is added to dilute blood which has been acidified with a few drops of hydrochloric acid, the chromoprotein is decomposed and the liberated hæmatin passes into the alcohol-ether layer.

As ordinarily prepared, "hæmatin" is a mixture of a ferric porphyrin, *methæm*, and a ferrous porphyrin, *hæm*. Hæmoglobin, itself, is a chromoprotein formed by the union of globin and hæm, and when oxidised to oxyhæmoglobin, the hæm component is converted into a labile form, oxy-hæm. Drastic oxidisers change the ferrous oxy-hæm into the ferric methæm, as occurs when methæmoglobin is prepared.

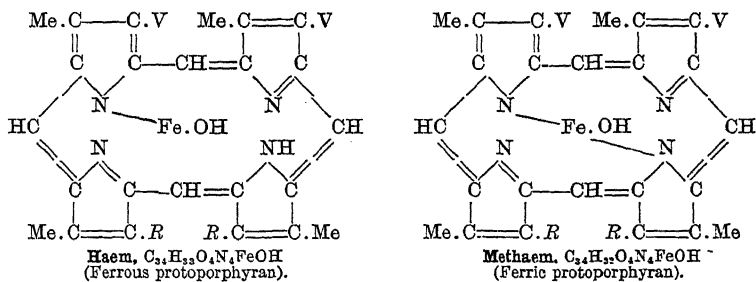
Hæmatin chloride, or **hæmin**, $C_{34}H_{32}O_4N_4FeCl$, a purple-brown crystalline salt that forms spontaneously in old blood clots, and may be prepared readily.

Mix a drop of blood, preferably defibrinated, with a drop of dilute (1 per cent.) sodium chloride on a glass slide. Allow to dry at room temperature. Add a couple of drops of glacial acetic acid to the dried blood, put on a cover slip, and warm gently over a small flame, until the red colour of the blood suddenly changes to dull violet. Allow the slide to cool, and examine microscopically, under the high power.

The individual crystals are dark brown elongated rhomboids or spindles, occurring singly or grouped as crosses or rosettes. Hæmin is insoluble in water, dilute acids, and neutral organic solvents, but dissolves with decomposition in alkalis, forming hæmatin, which may be precipitated in a pure condition by acidification of the solution.

Hæmin is important chemically as it is the starting-point for the study of the hæmatoporphyrin compounds.

Structure of the Hæms.—The iron-porphyrans, or hæms, like the other porphyrans are mono-metallic derivatives of a porphyrin. In hæm the iron is ferrous (divalent) and united to one of the pyrrole nitrogens, conventionally regarded as belonging to ring I. In hæmatin (methæm) and in hæmatin chloride, the iron is ferric (trivalent) and united to two nitrogen atoms in the parent porphyrin, protoporphyrin.



Hæmatin chloride is derived from methæm by replacement of the ferric $—OH$ by $—Cl$.

Hæmatoporphyrin, $C_{34}H_{38}O_6N_4$, an iron-free derivative of hæm, is obtained by the action of strong acids on hæmoglobin, or by dissolving hæmatin chloride in glacial acetic acid saturated with HBr . After four days, the mixture is diluted and the porphyrin precipitated by exact neutralisation. Hæmatoporphyrin is a dark violet powder, almost insoluble in water, but soluble in alcohol, alkalis, and concentrated sulphuric acid. It is a di-hydroxy derivative of **Protoporphyrin**, in which each vinyl side-chain, $—CH:CH_2$, has become $—CH_2.CH_2.OH$.

The acid solution has a very distinctive pair of absorption bands, one on either side of the D line. These may be demonstrated by the addition of 1–2 drops of undiluted blood to 10 ml. of concentrated sulphuric acid, and spectroscopic examination of the resulting purple mixture.

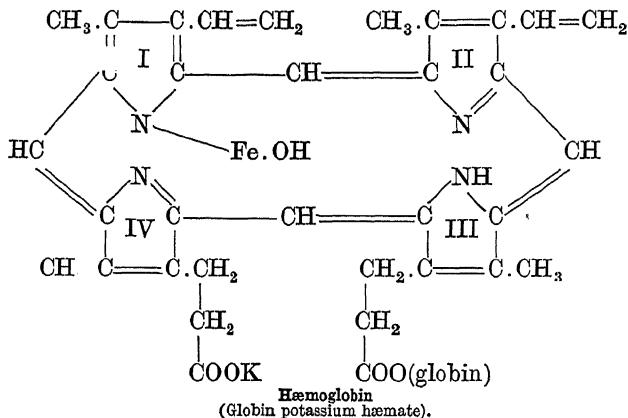
Derivatives of Protoporphyrin.—On heating with soda-lime, carbon dioxide is lost and *ætioporphyrin III* is formed. This porphyrin resembles one originally obtained from chlorophyll, and its preparation from the blood pigments is of great biochemical interest.

When protoporphyrin is oxidised in acid solution it is resolved into four pyrrole residues, the *hæmopyrroles*, from the study of which the structure of the original porphyrin has been confirmed.

The Phototoxic Properties of Hæmatoporphyrin.—Small intravenous injections of hæmatoporphyrin have no ill-effect on albino mice, rats, and guinea-pigs, as long as the animals are kept in the dark. On exposure to light, however, a severe dermatitis develops, often followed by œdema and death. The response persists for some weeks after sensitisation. It is not referable to foreign protein accompanying the injection, as animals are equally sensitive to autogenous hæmatoporphyrin.

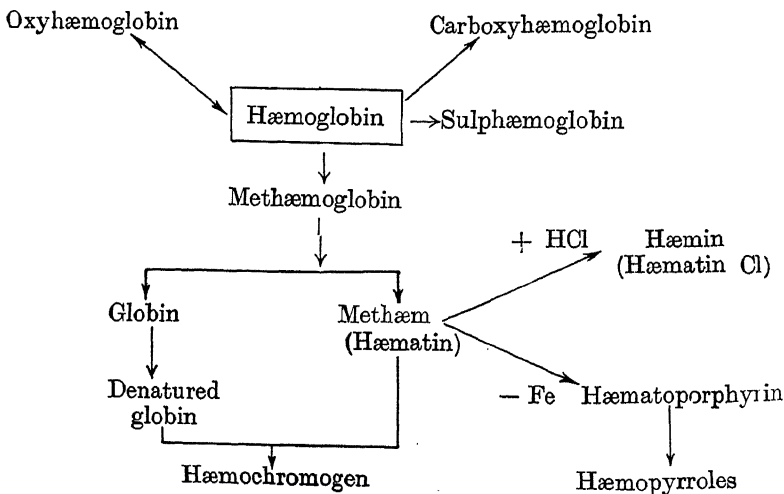
Structure of Hæmoglobin.—Hæmoglobin is a compound of the colourless, basic protein globin and the dicarboxy acid hæm, which

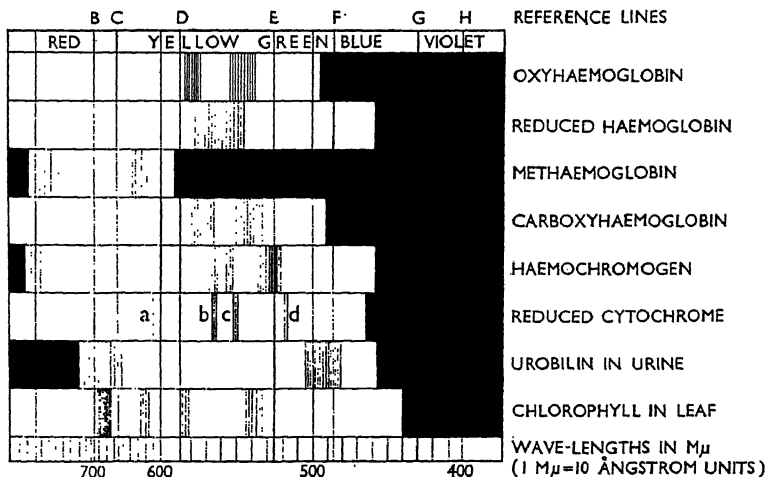
contains a carboxyl group at the end of one side-chain in rings III and IV of the parent protoporphyrin. One of these carboxyls is united to the globin, the other is united to potassium, since hæmoglobin occurs as a potassium salt within the red blood corpuscle. Hæmoglobin is only stable in neutral solutions, and relatively slight degrees of acidity or alkalinity are sufficient to resolve it into globin and hæm. As the constitution of globin is not known, it is only possible to represent hæmoglobin by a semi-structural formula.



In oxyhæmoglobin, the additional oxygen atom is assumed to be held loosely by a residual or semi-polar valency to the group $-\text{Fe} \cdot \text{OH}$.

Relationship of the Hæmoglobin Derivatives





Absorption Spectra of Pyrrole Pigments.

The D line of sodium has a wave-length of 587 r

THE CYTOCHROMES

Cytochrome.—In 1884, MacMunn observed that muscle and many other animal tissues possessed a sharply defined four-banded spectrum, which he attributed to a special pigment, *myohæmatin* or *histohæmatin*, so-called from its spectrographic resemblance to blood hæmatin. Hoppe-Seyler, the discoverer of hæmochromogen, opposed MacMunn's conclusion, and the work remained neglected until, in 1925, Keilin, using an intense system of illumination which allowed the examination of thick layers of tissue, showed that histohæmatin was universally distributed in all aerobic cells, and renamed the pigment *cytochrome*.

Cytochromes are present in all plant and animal tissues, and in aerobic unicellular organisms, such as yeasts and bacteria.

The spectrum shows four absorption bands, the position of which may differ slightly in cytochromes from different sources, but the general pattern of which shows a fundamental constancy. The position of maximum density of each band is: $a = 604.6 \text{ m}\mu$; $b = 566.5 \text{ m}\mu$; $c = 550.2 \text{ m}\mu$; $d = 521.0 \text{ m}\mu$. The c band being of greatest intensity is the one first sought.

Oxidation of cytochrome causes a disappearance of the characteristic spectrum, reduction, which may occur spontaneously in living tissue, causes its reappearance.

Cytochrome is a mixture of three different hæmochromogens, each

of which contribute two bands to the common spectrum: either *a*, *b* or *c*, and one component of the *d* band. Cytochromes from different sources differ in composition, as shown by the spectra.

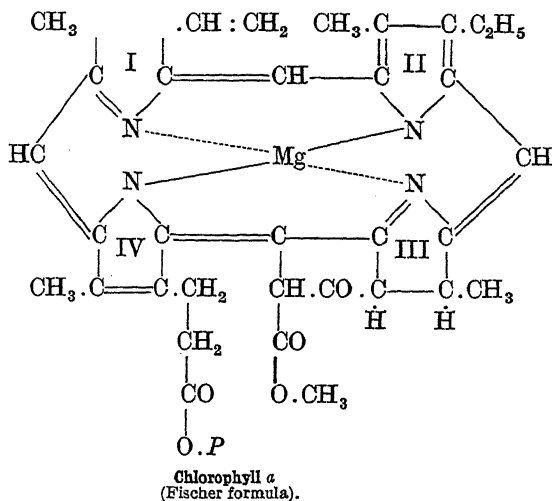
Function of Cytochrome C.—A general correspondence exists between the cytochrome content and the respiratory activity of a tissue, as measured by its oxygen consumption in presence of an oxidisable substrate. When dilute ($M/100$), cyanide is able to stop about 90 per cent. of the respiratory activity of most tissues, owing to its inhibitory effect on the enzyme, *cytochrome oxidase* (indophenol oxidase), which is necessary for the re-oxidation of cytochrome during the respiratory cycle. Observations such as this lead to the conclusion that cytochrome is "the main line of communication between oxygen itself and the substances which undergo oxidation in the cells" (Baldwin, 1936). The residual cyanide-stable respiratory activity is attributed to the presence of another respiratory catalyst, the "yellow enzyme" of Warburg, which differs from cytochrome in not being a hæm pigment and does not require the coaction of cytochrome oxidase.

CHLOROPHYLL

Chlorophyll, the green pigment of plant life, can be extracted easily from leaves by alcohol, acetone, ether, and similar organic solvents. The yield is about 1–2 gm. per kg. of fresh leaves, and 5–10 gm. per kg. of dried leaves. By subsequent extraction with a mixture of light petroleum and methyl alcohol, the pigment can be resolved into about 80 per cent. of chlorophyll *a* (soluble in petroleum) and about 20 per cent. of chlorophyll *b* (soluble in methyl alcohol). Both pigments are green, but they differ in their spectra in ethereal solution; chlorophyll *a* has a broad band in the red, 675–640 $m\mu$, and a narrower band in the orange, 615–605 $m\mu$, while chlorophyll *b* has a dark band to the right of the C line, 655–635 $m\mu$.

Structure.—Willstätter has shown that when chlorophyll is extracted from leaves by ethyl alcohol, an enzyme, *chlorophyllase*, present in the leaf, catalyses an alcoholysis of the pigment, splitting off phytyl alcohol, $C_{20}H_{39}.OH$, which is replaced by a $-C_2H_5$ group to give a mixture of *a* and *b* chlorophyllides. By alkaline hydrolysis, these chlorophyllides are converted into chlorophyllins, which are tricarboxy acids derived from a magnesium porphyrin, *ætiophyllin*. Treatment with acid removes the magnesium, leaving an *ætioporphyrin* closely related to the protoporphyrin found in hæmoglobin and hæm pigments. A large number of intermediate degradation compounds have been obtained by Willstätter, Fischer, and their

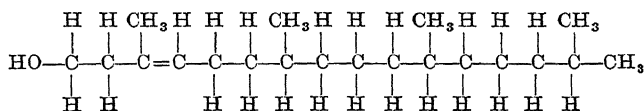
colleagues, who conclude that chlorophyll is the methyl phytyl ester of a tricarboxy magnesium porphyrin, the third carboxyl group of which is closed to form a lactam ring.



P represents the phytyl radicle, $C_{20}H_{39}$, which on hydrolysis of chlorophyll is liberated as phytyl alcohol.

In chlorophyll *b*, the $-CH_3$ group in ring II has been oxidised to $-CHO$.

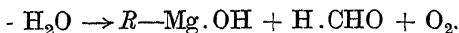
Phytol is a monohydroxy alcohol derived from the linear hydrocarbon hexadecane. Its origin and significance is unknown, but it is a probable precursor of the carotinoid pigments, and is a component of vitamin K.



Phytol, $C_{20}H_{39}.OH$
(2:6; 10:14-tetramethyl- Δ^{13}_{14} -16-hydroxy hexadecane).

Function of Chlorophyll.—The photosynthetic activity possessed by all green plants is dependent on chlorophyll, which, in presence of absorbed light, effects the conversion of carbon dioxide and water into the primitive carbohydrate unit and free oxygen. Neither the exact course nor the mechanism of the transformation is known, but it is suggested that the carbon dioxide first unites with the

magnesium atom in chlorophyll to produce an unstable acid carbonate, which is then reduced to formic acid or formaldehyde.



THE SIGNIFICANCE OF THE PORPHYRINS

(1) *Transport of the Pyrrole Nucleus from Plants to Animals.*—The porphyrin nucleus is both chemically and biologically stable, and once synthesised is not easily broken down either by plants or animals. Its history begins in the plant, where the pyrrole ring is synthesised, and appears in the amino acids tryptophane, proline and hydroxyproline, as well as in the hæm pigments and in chlorophyll. The ability of the higher animal to synthesise the pyrrole ring has not been established and it is known that one at least of the pyrrole-containing amino acids, tryptophane, is essential in animal nutrition. Consequently, it may be that the animal is dependent ultimately on the plant for its porphyrin units.

Chlorophyll is quantitatively an important constituent in the diet of herbivora, and a constant constituent in the human diet, and the suggestion has often been made that it is of value in nutrition.

Claims have been made by Abderhalden and others that chlorophyll has a therapeutic value in anæmia. There are, however, objections to this alleged nutritional importance. Chlorophyll is not attacked by any of the enzymes of the human alimentary tract, and its utilisation has to depend on bacterial decomposition. This may be significant in ruminants, where the parasitic factor in digestion is recognised, but is hardly so in man. It must not be forgotten that chlorophyll when it is degraded forms an important source of magnesium, in addition to providing porphyrins.

Marchlewski (1924) has shown that bilipurpurin, or cholehematin, a pigment present in the bile and biliary concretions of ruminants, is identical with *phylloerythrin*, a porphyrin pigment which he obtained by the biological degradation of chlorophyll or the acid hydrolysis of chlorophyllides. His discovery of phylloerythrin thus leads to the conclusion that the chlorophyll molecule may enter into the metabolic processes of herbivorous animals.

Bilirubin and biliverdin, the pigments of human bile, are derived from protoporphyrin, which, in turn, comes from hæmoglobin. Fission of the linkage between rings IV and I gives rise to biliverdin, which is reduced to bilirubin.

(2) *An Evolutionary Link between Plants and Animals.*—When investigators first realised that all flesh is grass, as far as its pigments are concerned, it was hoped that a path of chemical evolution might

be traced between the two kingdoms. Verne, for example, suggested that the hæm of animals may arise from vegetable chlorophyll, and, in consequence, the development of vertebrates was delayed until green plants had appeared in abundance. But, as Barcroft points out, the respiratory pigment cytochrome may be a much more ancient porphyrin than chlorophyll.

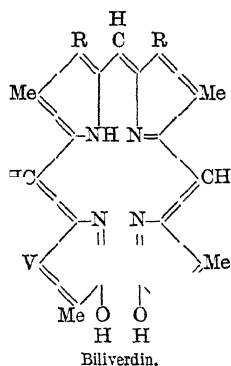
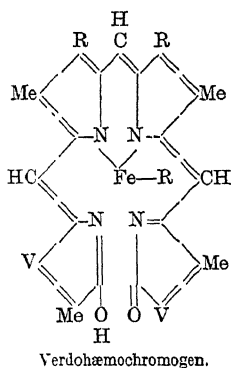
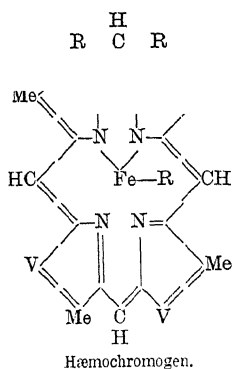
Yeast and bacteria, for example, grown on media entirely free from chlorophyll exhibit the spectral bands of cytochrome and hæm, which suggests that the chlorophyll has been evolved from hæm by the organism, and not hæm from chlorophyll.

Willstätter, who first elucidated the formula of chlorophyll, ascribes little biological significance to the hæmogoblin and chlorophyll relationship. Immediate utility and not biological tradition has determined the structure of each compound. But these conclusions were reached before the universal distribution of cytochrome was demonstrated.

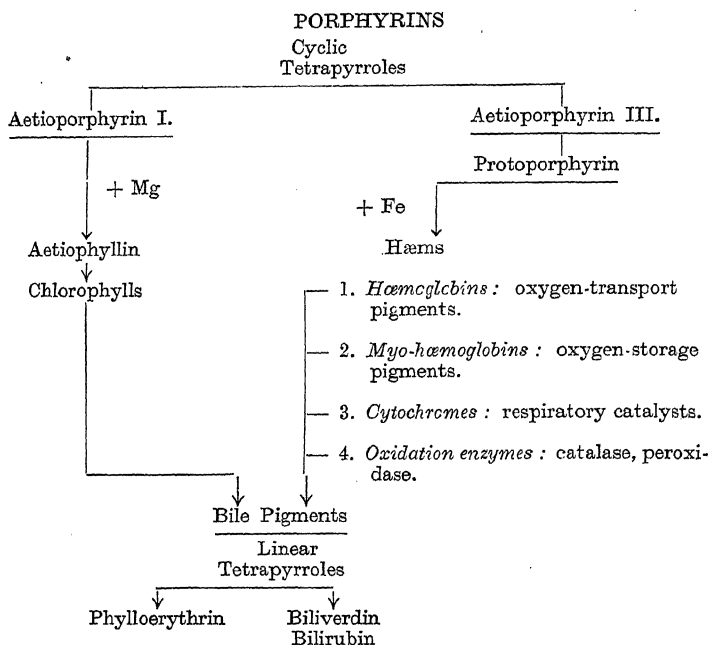
"Every detail points to something, certainly ; but generally to the wrong thing. Facts point in all directions, it seems to me, like the thousands of twigs on a tree. It's only the life of the tree that has unity and goes up—only the green blood that springs, like a fountain, at the stars."

G. K. CHESTERTON.

Conversion of Hæmoglobin Pigment into Bilirubin.—Lemberg has shown that oxidation of a hæmochromogen opens up the tetrapyrrole ring to form a green verdohæmochromogen, which is a linear tetrapyrrole and convertible into biliverdin by removal of the iron atom by acids.



Subsequent reduction of the biliverdin forms bilirubin.



CAROTINOIDS

Carotinoids are fat-soluble hydrocarbon pigments widely distributed in low concentrations throughout the animal and plant kingdoms. They are orange or red in colour, crystallisable, freely soluble in light petroleum, chloroform and similar fat-solvents, but insoluble in water. They are easily bleached by oxidation, and give a deep blue colour in presence of concentrated sulphuric acid (the carotinoid reaction). Since they accumulate in oils and fats, they are also termed *lipochromes*. Carotinoids impart the typical colour to egg-yolk, the corpus luteum of the ovary, butter fat, liver oils, carrot, turnip, maize, tomato, "the sere and yellow leaf," and many fruits and flowers.

History.—The group name is derived from that of the first member discovered, *carotin* or carotene, which, in 1831, was isolated from carrots, and subsequently was found to occur along with another yellow pigment, *xanthophyll*, in all chlorophyll-containing foliage. Between 1906 and 1914, Willstätter isolated nearly a dozen different carotinoids, and Palmer showed that the pigment of milk and butter fat is carotene derived from the diet of the cow (1922). The pigment of egg-yolk, originally termed "lutein," was

shown to be a mixture of carotene and leaf xanthophyll, the latter being now re-named *lutein*.

PLANT CAROTINOIDS

In Order of Adsorption from Petroleum Solutions

Name.	Formula.	Chief Sources.	Adsorbent.
<i>Alcohols :</i>			
Flavoxanthin . . .	$C_{40}H_{56}O_3$	Buttercup	$CaCO_3$
Violaxanthin . . .	$C_{40}H_{56}O_4$	Pansy	"
Taraxanthin . . .	$C_{40}H_{56}O_4$	Dandelion	"
Lutein . . .	$C_{40}H_{56}O_2$	Green leaves	"
Zeaxanthin . . .	$C_{40}H_{56}O_2$	Maize	"
<i>Ketone :</i>			
Rhodoxanthin . . .	$C_{40}H_{50}O_2$	Rose fruit	"
<i>Hydrocarbons :</i>			
γ -Carotene . . .	$C_{40}H_{56}$	Carrot	$Al(OH)_3$
β -Carotene . . .	$C_{40}H_{56}$	Carrot	"
α -Carotene . . .	$C_{40}H_{56}$	Carrot	"

Carotene, $C_{40}H_{56}$.—Fresh carrot contains about 0.1 per cent. of carotene, and fresh grass contains about 0.01 per cent. of carotene and 0.02 per cent. of lutein (xanthophyll). The pigment is easily obtained by extracting dried carrot scrapings or dried leaves with light petroleum (which does not extract the chlorophyll). The extract is evaporated in a dish at room temperature, and the residue freed from lipides by rinsing with small quantities of the solvent. The carotene fraction remains as a crop of dark red micro-crystals, which by chromatographic adsorption, can be resolved into three isomers.

α -Carotene, m.p. 175° , $[\alpha]_D = +323^\circ$, forms brilliant rhomboidal crystals, copper-red in colour.

β -Carotene, m.p. 185° , $[\alpha]_D = 0^\circ$, resembles the α -form, but is slightly less soluble. β -Carotene occurs almost pure in spinach and in red pepper (paprika). The carotene fraction from palm-nut oil contains about 60–70 per cent. of the β -form, the rest being α -carotene.

γ -Carotene is rare in higher plants, but has been found in the acid-fast bacteria. It differs from the other isomers in having only one ring in its molecule.

Lycopene, $C_{40}H_{56}$, the colouring matter of tomatoes, differs from the carotenes structurally in being a non-cyclic hydrocarbon.

Chromatographic Analysis

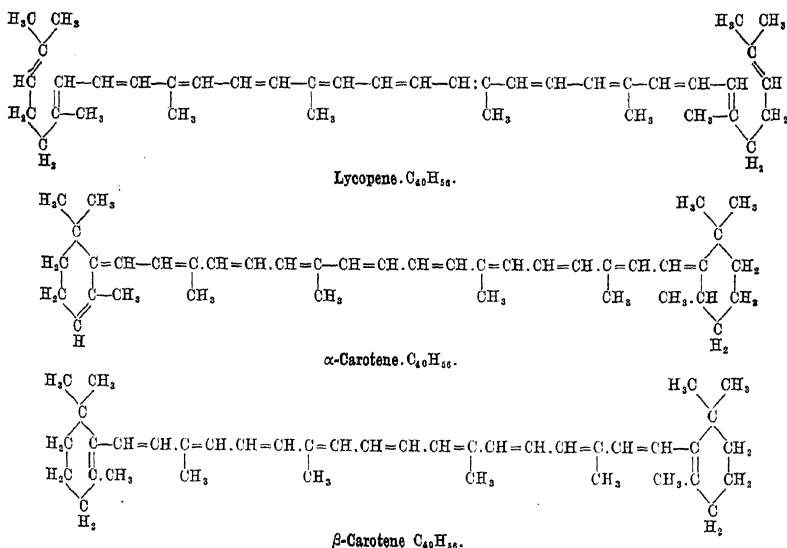
The Russian botanist, Tswett, found in 1906 that a tube packed with powdered calcium carbonate could be used to separate pig-

ments from solution in non-polar solvents, such as light petroleum. When the solution was allowed to flow down the tube, the pigments tended to be adsorbed at different stages, thus producing a striated *chromogram*. Twenty years later, Kuhn and his colleagues applied this method to the resolution of the carotinoids, and were able to fractionate crude carotene into (i.) an optically inactive β -carotene, and (ii.) a dextro-rotatory α -carotene, the latter being adsorbed below the former. A narrow zone of pigment above the β -carotene layer proved to be a third isomer, γ -carotene, 34 mg. of which were obtained from 35 gm. of crude carotene, which, in turn was got from 300 kg., or about 6 cwt., of raw carrots.

Chromatographic adsorbability is increased by the presence of hydroxyl groups and unsaturated linkages in the adsorbent, and thus affords information about the structure of the compounds.

Structure of the Carotinoids

On reduction, lycopene was found by Karrer to accept 26 hydrogen atoms and give rise to a paraffin hydrocarbon, $C_{40}H_{82}$, which led him to conclude that the pigment is a linear compound containing 13 unsaturated linkages. Carotene, although an isomer of lycopene, was found by Zechmeister to accept only 22 atoms of hydrogen, from which he concluded that it contained two cyclic groups separated by an unsaturated chain. These groups were subsequently identified as being derived from β -ionone, a constituent of the essential oil of the violet.



The hydrocarbon carotinoids differ from each other in the arrangement of the terminal groups, both of which may be open, as in lycopene, or both closed to form 6-membered rings, as in α - and β -carotene.

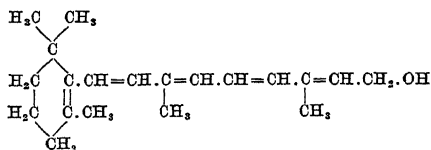
The xanthin carotinoids are ketones or alcohols derived from carotenes by substitution of H in the terminal rings.

Vitamin A

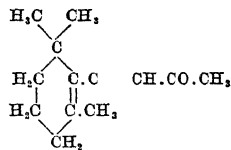
In 1919, Steenbock suggested that the fat-soluble vitamin A was related in some way to the carotinoids because of the similarity of distribution in natural sources. Inadequate knowledge of the properties of the vitamins delayed confirmation of the hypothesis, until, in 1929, von Euler showed that although carotene was not identical with vitamin A, it could replace the vitamin in the diet. Moore subsequently demonstrated that the pigment was transformed into the vitamin within the liver of the animal. Meanwhile, the carotenes had been resolved into their isomers, and it was found that β -carotene had twice the provitamin potency of α -carotene. Since the formula of vitamin A was known approximately to be $C_{20}H_{29}.OH$, it was suggested that the vitamin was hydroxy-*semi*- β -carotene, two molecules of which were derived from one molecule of β -carotene by hydrolytic fission.

α -Carotene, γ -carotene, and kryptoxanthin each contain a *semi*- β -carotene residue, and can give rise to one molecule of vitamin A. Consequently, the three carotenes and kryptoxanthin are provitamins of A, and β -carotene is taken as the international standard for comparison (p. 243).

Structurally, vitamin A contains a β -ionone nucleus carrying a side-chain with four unsaturated linkages and a terminal primary alcohol group. A related form, vitamin A_2 , $C_{21}H_{31}O$, occurs in livers of fresh-water fishes. It differs from A, in having an additional $-CH :$ link in the side-chain.



Vitamin A.



β -Ionone.

Animal Carotinoids.—The carotinoids found in higher animals are usually plant carotinoids derived from the diet; the grass of the field being the principal source of the provitamins necessary for human life. Although the carotene : xanthophyll ratio in grass is 1 : 2, both cows and horses preferentially absorb the carotene to the exclusion of the xanthophyll. Birds accumulate xanthophyll almost exclusively, and it reappears in the egg-yolk, body fat and

plumage, a fact known empirically by canary breeders. Man absorbs carotene and xanthophyll with equal facility, and both pigments occur in human plasma. Milk fat, the commonest dietetic source of carotene, contains from 1 to 20 parts per million, depending on the nature of the food consumed.

Among the lower animals, carotinoids occur as special body pigments. The shells of the lobster and other crustacea contain a blue chromoprotein, which on hydrolysis by boiling liberates *astacene*, $C_{40}H_{56}O_4$, a tetra-keto- β -carotene. Astacene is the typical pigment of echinoderms, gold-fish skin and the flesh of salmon. *Violerythrin*, $C_{40}H_{56}O_6$, is the carotinoid that as an ester, *actinoerythrin*, imparts the brilliant colour to sea anemones.

Biogenesis of the Carotenoids.—According to Karrer, the alcohol *phytol*, $C_{20}H_{39}.OH$, which forms about one-third of the chlorophyll molecule, is the parent substance of the carotinoids. Condensation of two phytol residues would in theory yield lycopene, from which the other carotinoids could arise by closure of the terminal groups to form rings. The symmetry of the carotinoid molecule suggests an origin from such a precursor.

The structural unit of both phytol and the carotinoids is *isoprene*, $H_2C=C(CH_3)-CH=CH_2$, which presumably arises during the photosynthesis of carbohydrates.

Analytical Reactions of the Carotinoids

Examine microscopically a thin section of carrot. In the cortical region minute needle-shaped orange crystals can be seen in the cells. These are stearate coloured by carotene. Cover the preparation with a glass slip, and carefully instil a drop of concentrated sulphuric acid. The crystals develop a deep blue colour.

Apply the following tests to a specimen of fish-liver oil diluted 1 : 10 with chloroform, or to a solution of carotene in chloroform.

(1) *Ferric Chloride Test.*—Addition of a few drops of 1 per cent. ferric chloride to 5 ml. of the solution produces a bright green colour owing to reduction of the iron to the ferrous state.

(2) *Sulphuric Acid Test.*—Careful addition of a couple of drops of concentrated sulphuric acid to a water-free solution of a carotinoid produces a transient blue-violet colour, which is very marked if the mixture contains vitamin A.

(3) *Antimony Trichloride Test.*—When 2–5 ml. of a chloroform solution of a carotinoid are treated with an excess of a 30 per cent. solution of antimony trichloride in chloroform a stable blue colour develops.

A similar colour is given by vitamin A (Carr-Price test), but the

vitamin blue can be distinguished by the possession of an absorption band in the region of 562–583 $m\mu$, which is not shown by any of the carotinoids. A maximum absorption band is shown by vitamin A_1 at 617 $m\mu$. ; and by vitamin A_2 at 693 $m\mu$.

LYOCHROMES, OR FLAVINS

It has been known for many years that animal tissues display a greenish fluorescence on exposure to ultra-violet radiation. In 1929, Ellinger, during an exploration by means of his "intra-vital microscope," observed that the fluorescent pigment was concentrated chiefly in the liver and upper renal tubular epithelium of all species of animals examined. Using the fluorescence as a guide, he succeeded in extracting and purifying the compound by absorption on Fuller's earth, and subsequent elution with aqueous pyridine. Milk whey proved to be a good source of the pigment, which was eventually obtained in crystalline form, and was termed a *lyochrome*, since it was water-soluble, thus differing from the fat-soluble lipochromes, or carotinoids.

Meanwhile, two groups of investigators working along different lines had also recognised the existence of this new type of pigment. Warburg and Christian had shown that their "yellow ferment" or respiratory catalyst, originally obtained from brewer's yeast, on hydrolysis liberated a lyochrome apparently identical with one obtained by Ellinger; and Kuhn, during a study of the vitamin B complex, found that one member, vitamin B_{12} , was itself a lyochrome.

In addition, lyochromes were obtained from a variety of animal and plant sources, and were named by affixing the termination *flavin*, to indicate the yellow colour of the pigment: *Lactoflavin*, from whey; *ovo-flavin*, from egg-white; *hepatoflavin*, from liver; *renoflavin*, from kidney; *uroflavin*, from urine; *maltoflavin*, from malt; and *zymoflavin*, from yeast.

Definition.—Lyochromes are nitrogenous pigments derived from iso-alloxazine, and characterised by: (1) solubility in water and insolubility in fat-solvents; (2) yellow colour in solution, and orange-red colour in crystalline form; (3) greenish-yellow fluorescence in neutral aqueous solutions, the fluorescence being extinguished by addition of acid or alkali; (4) stability to oxidising agents; and (5) reversible reduction to leuco-compounds.

The concentration of these lyochromes in their natural sources is very low. Fresh liver or kidney contains 10–20 mg. per kg., whey contains up to 80 mg. per litre, consisting of a mixture of related lactoflavins, *a*—*d*.

Riboflavin, lactoflavin or vitamin B_2 , $C_{17}H_{20}O_6N_4$, the most

important of the lyochromes, according to Ellinger occurs in free and bound forms.

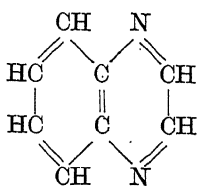
(1) *Flavoprotein*, Warburg's "yellow enzyme," is a widely distributed natural pigment which acts as a respiratory carrier in the oxidation of hexose phosphates, malate and alcohol. It is a conjugated protein, the prosthetic flavin of which was discovered by Szent-Györgyi, who named it "cytoflave" prior to its identification with vitamin B₂. In the absence of the protein component, the flavin has no respiratory activity.

(2) *Flavopurines*.—Lactoflavins *a*, *b* and *c* of milk, in which the flavin is united to a purine derivative.

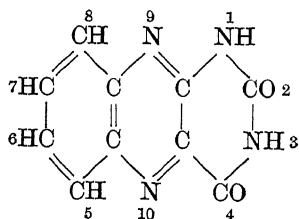
(3) *Phosphoflavins*.—Co-carboxylase, a phosphoric ester of riboflavin. Cytoflave, the phosphoric ester of riboflavin, forming the prosthetic group in flavoprotein.

(4) *Free Flavins*.—Principally riboflavin, which is identical with the ovoflavin, hepatoflavin, and lactoflavin *d* of various workers.

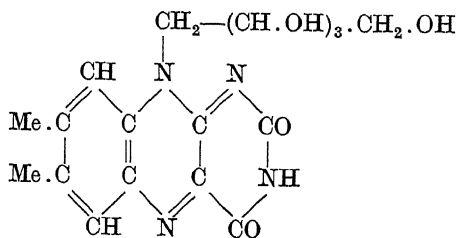
Structure of the Flavins.—When riboflavin is irradiated in alcohol, a sugar, D-ribose, is split off, leaving a crystallisable residue, *lumichrome*. Alkaline hydrolysis of lumichrome yields urea and a dimethyl benzpyrazine, which suggests the presence of three rings in the original flavin, one of which has yielded the urea. This has led to the *iso*-alloxazine formula now adopted for the lyochromes, according to which lumichrome is the 6 : 7-dimethyl derivative.



Benzypyrazine.



iso-Alloxazine.



Riboflavin, vitamin B₂
(6 : 7-dimethyl-9-D-ribose *iso*-alloxazine).

GENERAL REFERENCES

- ANSON, M. L., and A. E. MIRSKY (1930), "Hæmoglobin, the heme pigments and cellular respiration." *Physiol. Rev.*, **10**, 506.
- BARCROFT, J. (1924), "Significance of hæmoglobin." *Physiol. Rev.*, **4**, 329; (1925), **5**, 596.
- BARCROFT, J. (1928), "Respiratory Function of the Blood," Vol. II. Cambridge.
- BOGERT, M. T. (1938), "Carotenoids." *Organic Chemistry*, Ed. Gilman, **2**, 1138.
- ELLINGER, P., and W. KOSCHARA (1934), "The lyochromes," *Nature*, **133**, 553.
- EULER, H. (1936), "Water-soluble vitamins." *Ann. Rev. Biochem.*, **5**, 359.
- FISCHER, H. (1937), "Chlorophyll," *Chem. Rev.*, **20**, 41.
- HEILBRON, I. M., and A. GILLAM (1937), "Pigments associated with the fatty tissue of plants and animals." *Nature*, **139**, 612, 657.
- KEILIN, M. D. (1936), "Intracellular Respiration." *Bul. Soc. Chim. Biol.*, **18**, 96.
- KUHN, R. (1935), "Plant pigments," *Ann. Rev. Biochem.*, **4**, 490.
- KUHN, R. (1935), "Flavins," *Bul. Soc. Chim. Biol.*, **17**, 905.
- LEDERER, E. (1934), "Les carotinoids des plants." Paris.
- LINSTEAD, R. P. (1936), "The porphyrin group." *Chem. Soc. Ann. Rep.*, **32**, 359.
- MACMUNN, C. A. (1914), "Spectrum Analysis Applied to Biology and Medicine." London.
- PALMER, L. S. (1922), "Carotinoids and Related Pigments." New York.
- ROCHE, J. (1936), "Animal pigments," *Ann. Rev. Biochem.*, **5**, 463.
- ROCHE, J. (1936), "La biochemie générale et comparée des pigments respiratoires." Paris.
- SMITH, J. H. C. (1937), "Plant pigments," *Rev. Ann. Biochem.*, **6**, 489.
- TURNER, E. E. (1935), "Lactoflavin," *Ann. Rep. Chem. Soc.*, **32**, 354.
- TURNER, W. J. (1937), "Studies on porphyria." *J. Biol. Chem.*, **118**, 519.
- WALDSCHMIDT-LEITZ, E. (1933), "Chemical nature of enzymes." *Science*, **78**, 189.
- ZECHMEISTER, L. (1934), "Carotinoide." Berlin.

CHAPTER XIII

CATALYSTS

“Catalysis is one of the most significant devices of nature, since it has endowed living systems with their fundamental character as transformers of energy, and all evidence suggests that it must have played an indispensable part in the living universe from the earliest stages of evolution.”

F. G. HOPKINS.

ORGANIC life is manifest in a continuum of chemical reactions, the course and rate of which are determined by the presence of various catalysts. These agents usually reveal themselves by activating or rendering unstable certain compounds or substrates, and thereby bringing about chemical changes.

Chemical Reactions.—These involve the redistribution of atoms and the reconstruction of molecules. They exhibit (i.) *direction*, (ii.) *rate*, and (iii.) *extent*.

The *direction* of a reaction is expressed by the change :—

Reactants \longrightarrow End-products.

The *rate* of a reaction is defined as the quantity of substance transformed (removed or produced) in unit time.

The *extent* of a reaction is measured by the percentage change in the concentrations of the constituents.

While the three properties of direction, rate, and extent are dependent primarily on the chemical nature of the reactants, they are liable to be modified in various ways, of which the following are the most important :—

- (1) *Concentration of Reactants.*—At constant temperature, the rate of a reaction at a given instant is proportional to the product of the concentrations of the reactants at that instant.

$$\text{Rate} \propto [a] [b] \dots [z],$$

where $[a]$, $[b]$, $[z]$ denote the concentration, usually expressed in gram-molecules per litre, of each reactant (Law of Mass Action).

- (2) *Concentration of End-products.*—Reactions are retarded both by decrease in reactants and increase in end-products,

- ~ until finally a state of equilibrium is reached when the composition of the mixture remains constant.
- (3) *Temperature*.—The rate of a simple chemical reaction is increased approximately two-fold for each rise in temperature of 10°C ., within critical limits.
- (4) *Catalysis*.—Many reactions are highly susceptible to the influence of specific agents, or catalysts, which cause an enormous increase in the reaction rate without being used up in the process. Negative catalysts, or reaction inhibitors, are also known.

Catalysed reactions are characterised by :—

- (1) *Survival of the Catalyst*.—Unlike other reactants it is not necessarily removed by the reaction.
- (2) *Continuity of Effect*.—A minimal amount of the catalyst is able to affect the transformation of an indefinitely large amount of reactant.

For example, a highly active sucrase has been prepared that can hydrolyse ten times its weight of sucrose per second for an indefinite time, provided the conditions are kept favourable.

- (3) *Independence of Equilibrium*.—The final state of a chemical reaction is independent of the way in which the state has been obtained. Catalysts do not, as a rule, alter the equilibrium value, and hence cannot have added to the energy of the system.

Definitions of a Catalyst.—(1) "A catalyst is a substance that accelerates a reaction but cannot induce a reaction" (Bodenstein, 1902).

(2) "In a catalysed reaction the chemical composition of one reactant is the same as that of one of the products—this substance is the catalyst" (Falk, 1922 ; Northrop, 1926).

The definition of a catalyst as a reaction-accelerator when first proposed by Ostwald stimulated research into the kinetics of catalysed reactions. Falk's definition is less ambitious and more precise, and furthermore avoids the objection that many reactions susceptible of catalysis do not appear to take place at all in the absence of the catalyst.

Examples are the stability of sucrose and of urea at ordinary temperatures, provided the solutions be kept sterile, a precaution generally overlooked by earlier workers.

(3) "A catalyst is a substance which enables certain molecules to undergo chemical changes on receiving a critical energy that is less than they would require in the absence of the catalyst" (Hughes, 1933).

Biological Catalysts.—The catalysts manufactured and used by living organisms appear in two forms: (a) *catalytic surfaces*, such as occur inside cells; (b) *catalytic solutes*, such as are found dissolved in secretions and tissue extracts. The distinction between the two forms is not final; by disintegration, catalytic surfaces may be removed as solutes, and, conversely, inside the cell many catalytic solutes may be concentrated in particular regions.

Catalytic solutes may be subdivided into (i.) *non-colloidal* catalysts, and (ii.) *colloidal catalysts* or enzymes. The first subgroup includes soluble catalysts of low molecular weight, such as electrolytes, glutathione and ascorbic acid; the second group includes the vast assortment of enzymes that in variety and importance dominate the biochemistry of catalysis.

(i.) *Non-colloidal Catalysts*

H-ions.—Catalyse the hydrolysis of disaccharides and esters, and many other organic reactions.

HO-ions.—Catalyse the oxidation of polyphenols, the isomeric transformation of monosaccharides, the conversion of atropine into hyoscyamine, and other changes.

I-ions.—Catalyse the decomposition of hydrogen peroxide, and many oxidations.

(ii.) *Colloidal Catalysts*

Metallic Sols.—Metals in colloidal form modify many chemical reactions, usually those concerned with oxidation or hydrogenation, as, for example, the use of nickel in the hardening of oils into fats.

Enzymes.—Biological catalysts obtainable in soluble, colloidal forms.

Biological, because they are produced by living organisms.

Soluble, because they occur in secretions or can be extracted from tissues by means of aqueous solvents.

Colloidal, because the molecular dimensions are greater than $1\text{ m}\mu$, and the particles in consequence will not pass through collodion or parchment membranes.

Enzymes are defined by Waldschmidt-Leitz (1936) as: Catalysts of a definite organic nature with a specific activity, formed only by living cells but acting independently of living cells.

(iii.) *Insoluble Catalysts*

Catalytic Surfaces.—These may be specialised areas of the cell-wall mosaic or may be enzymes immobilised in various ways on the cyto-skeleton. Willstätter (1933) has shown that in addition

to the soluble, or *lyo*-enzymes, a class of bound or *desmo*-enzymes exists which can only be separated after the parent tissues have undergone autolysis (self-digestion), or have been extracted with organic solvents.

The History of Enzyme Chemistry.—Four spontaneous natural changes have been known to man ever since he first acquired the power of rational observation. They are : (i.) the alcoholic fermentation of sugars ; (ii.) the lactic fermentation, or souring, of milk ; (iii.) the acetic fermentation of wine ; and (iv.) the ammoniacal fermentation of urine. Each is due to the growth of a microscopic organism and its attack on a particular substance or *substrate*. The mechanism of these natural fermentations remained completely obscure until the beginning of the last century.

(1) In 1830, Dubrunfaut found that malt extract could convert starch paste into sugar in a manner comparable to the action of a strong acid, as shown previously by Kirchoff (1815). In 1833, Payen and Persoz separated the active amylolytic principle from malt extract by addition of excess of alcohol. This precipitate, the first true enzyme isolated in the crude state, they named *diastase*, and compared it to one of the unknown natural "ferments" that brought about the souring of milk or wine. The wide distribution and importance of these ferments was realised, and Berzelius introduced the term *catalysis* to describe the changes that they brought about.

(2) Between 1850 and 1870, Pasteur showed that the natural fermentations were invariably due to the growth of micro-organisms, called by him the "organised ferments." Non-living agents, such as the pepsin of gastric juice or the diastase of malt extract were called the "soluble or unorganised ferments."

(3) Confusion having arisen as to the meaning of "ferment," Kühne, in 1870, introduced the term *enzyme* to describe biological catalysts irrespective of their origin.

(4) In 1896, Ostwald defined a catalyst as the accelerator of a chemical reaction. This stimulated research into the physical chemistry of enzymes, and many equations were obtained connecting speed of zymolysis with concentration of catalyst, substrate, and end-products.

(5) Since 1900, enzyme research has developed to such an extent that each single enzyme or a single property of a class of enzymes has proved sufficient to engage the attention of a group of investigators. Important dates are : 1926, crystallisation of urease (Sumner) ; 1932, discovery of flavin enzymes (Warburg) ; 1933, isolation of the co-enzymes ; 1935, isolation of virus protein (Stanley).

Properties of Enzymes.—In addition to the general endowments of all catalysts, namely, (i.) power of survival, (ii.) continuity of effect, and (iii.) independence of equilibrium, the enzymes display special properties of their own, including :—

- (1) Colloidal characteristics.
- (2) High specificity.
- (3) Sensitivity to temperature.
- (4) Sensitivity to H-ion concentration.
- (5) Sensitivity to electrolytes.
- (6) Sensitivity to specific factors, including co-enzymes, activators and toxins.

While many non-colloidal catalysts are modified by one or more of these factors, enzymes as a class are highly sensitive to all of them.

Colloidal Characteristics.—In secretions and extracts, enzymes are closely accompanied by inert colloids, usually proteins, the removal of which is the greatest problem in purification. However, even when in the highest state of purity yet attained, enzymes themselves are colloidal solutes of low diffusibility and high molecular weight.

The colloidal state is determined by the size of the solute particle in reference to the solvent. For aqueous solvents, the range is $1\text{ m}\mu$ – $100\text{ m}\mu$, above which the particles are too large to form stable dispersions. The size of the particles may be found by various methods, including: (i.) ultra-filtration through membranes of known porosity, (ii.) rapidity of diffusion, and (iii.) fractionation by centrifuge accompanied by observation of diffusion rate.

Approximate Molecular Weight and Diameter of Typical Enzymes

Enzyme.	Molecular Weight.	Diameter in $\text{m}\mu$.
Pepsin	37,000	4.2
Trypsin	34,000–45,000	4.4–5
Emulsin	37,700	4.2
Saccharase	44,900	5.0

($\text{m}\mu$, the millimicron, is one millionth of a millimetre.)

These data show that enzyme particles are of the same order of magnitude as the heat-coagulable proteins.

Specificity.—By specificity of an enzyme is meant the restriction of the catalytic effect to one particular substrate or a group of substrates. Three types of specificity are recognised :—

- (a) *Absolute specificity of attack*, when the enzyme acts only on

one substrate. The decomposition of urea by *urease* is an example.

- (b) *Relative specificity of attack*, when an enzyme acts on two or more substrates, usually with different intensities. The hydrolysis of esters by *lipase* is an example.
- (c) *Specificity of reaction path*, when an enzyme decomposes a substrate in a particular way, although the final products may be the same as those obtained by the aid of other catalysts. Thus, when the trisaccharide raffinose is hydrolysed by yeast *saccharase*, it yields fructose and melibiose. When hydrolysed by *emulsin* it liberates sucrose and galactose.

Temperature Sensitivity.—This is shown by two important characteristics—heat-inactivation and heat-acceleration. The irreversible inactivation by heat is used to distinguish enzymes from non-enzymatic catalysts, and is one of the means of detecting enzymes.

All known enzymes are inactivated, or "killed," by being boiled for a few minutes in an aqueous solution.

Sensitivity to temperature is increased by purification and by certain conditions of H-ion and electrolyte concentrations, hence the temperature of "thermal inactivation" is not sufficiently precise to be used for identifying a particular enzyme.

Heat-inactivation is ascribed to irreversible changes in the colloid enzyme comparable with the heat-coagulation of higher proteins.

Heat-acceleration is shown by the existence of an optimal temperature range for every reaction, although the limits of the range are modified by various factors, including the gradual inactivation of the enzyme by prolonged heating.

Sensitivity to H-ion Concentration.—All enzymes display a region

Optimal H-ion Values for Typical Enzymes

Enzyme.	Source.	Substrate.	Optimal pH.
Pepsin	gastric mucosa	proteins	1.5-2.5
Peroxidase	plant roots	hydrogen peroxide	3
Phosphatase	<i>Aspergillus</i>	glycerophosphate	3.6
Zymase	yeast	glucose	4.5-6.5
Lipase	gastric mucosa	fats	5.5-8.6
Amylase	malt	starch	5.2
Amylase	pancreas	starch	6-7
Urease	Soy bean	urea	7-8
Trypsin	pancreas	proteins	8-9
Phosphatase	cartilage	pyrophosphates	7.5
Phosphatase	cartilage	phosphoric esters	9.0-9.2

of optimal H-ion concentration, which varies from about pH 1.5 for pepsins up to about pH 10.0 for trypsin and mammalian phosphatase. The majority act best within the range pH 4.5–pH 7.5.

As well as optimal pH values there are maximal and minimal limits beyond which the enzyme does not act. Exposure to these extremes is often marked by destruction of the catalyst in a manner comparable with thermal inactivation.

Sensitivity to Activators.—Many enzymes are powerfully affected by specific ions or simple solutes, which either accelerate or inhibit the reaction. Activators are divided provisionally into *promoters*, that increase the rate at which the reaction starts; and *protectors*, that prolong the rate by retarding the spontaneous inactivation of the enzyme.

Promoters.—In low concentration, Mg ions activate bone phosphatase; Ca ions activate pancreatic lipase; Co^{++} and Mn^{++} activate arginase; Cl ions probably are necessary for amylase; Fe^{+++} participates in various oxidation systems.

Cyanide, hydrogen sulphide and other thiol ($-\text{SH}$) compounds, including cysteine and reduced glutathione, in low concentration, activate papain, cathepsin, urease and other enzymes.

Protectors.—Agents capable of neutralising toxic contaminants or by-products during zymolysis. Thus, α -amino acids activate urease, arginase and probably other enzymes.

Co-enzymes.—Heat-stable organic compounds naturally associated with complex enzyme systems, and necessary for zymolysis. They act as carriers of hydrogen, phosphate, or other intermediate reactants in the system.

Co-dehydrogenase I, co-enzyme I or co-zymase, obtainable from extracts of yeast, muscle, liver and kidney, is the co-enzyme of sugar fermentation, and acts with the dehydrogenases of hexose diphosphate, malate, and alcohol, and also with the lactate dehydrogenase of muscle.

Co-dehydrogenase II, co-enzyme II (Warburg), obtained from yeast and red blood cells, acts with hexose monophosphate dehydrogenase.

Co-enzyme of D-amino acid oxidase.—*Co-carboxylase*, the pyrophosphoric ester of vitamin B_1 .

Glutathione acts as a co-enzyme for glyoxalase and for the intracellular proteinases of animal tissues.

Inhibitors and Inactivators.—These include ions of heavy metals, especially silver and mercury, aldehydes and nitrous acid, which attack amino groups; amino compounds, which combine with aldehyde groups; relatively weak oxidising agents; reducing agents in high concentration, and the more specific *anti-enzymes* found in blood and tissues. Inactivation may be reversible or irreversible, and varies greatly with different types of enzymes.

CLASSIFICATION OF ENZYMES

Enzymes are classified according to the compound, or **substrate**, they act upon, the name of each enzyme or enzyme type being

derived by attaching the suffix **ase** to the name of the substrate. Thus, *esterases* activate esters, and *urease* decomposes urea. The older names, such as pepsin and trypsin, are still retained, partly because of long established usage, and partly because some of them apply to groups of closely related catalysts.

A. HYDROLASES

Enzymes causing hydrolytic decomposition of the substrate by attacking, or *activating*, specific linkages.

(1) **Esterases**.—Attack the ester linkage, —CO.O—CH= or A—O—CH= , where *A* is an acid radicle. *Phosphatases* form an important class of esterase, and activate the linkage R—O—PO(OH)_2 , liberating phosphoric acid.

(2) **Carbohydrases**.—Convert higher carbohydrates into simple sugars.

(a) **Polysaccharidases** (Polyases).—Depolymerise polysaccharides.

(b) **Saccharidases** (Hexosidases and Pentosidases).—Attack the oxygen linkage, —CH(OH)—O—CH= , in compound saccharides.

(3) **Proteases**.—Attack the peptide linkage, —CO—NH— , in proteins.

(a) **Proteinases**.—Preferentially attack central peptide linkages.

(b) **Peptidases**.—Only attack terminal peptide linkages.

(4) **Aminases**.—Attack the amino group or the imino linkage, =CH—NH— , in amino and related compounds.

B. OXIDO-REDUCTASES

Enzymes activating oxidation-reduction systems, one component of which is the substrate.

(1) **Dehydrogenases** (Dehydrases).—Transfer hydrogen from the substrate to a hydrogen acceptor, thus effecting an indirect oxidation.

(2) **Oxidases**.—Activate the oxygen linkage, —O—O— , in molecular oxygen, peroxides and other oxygen donators.

C. DESMOLASES

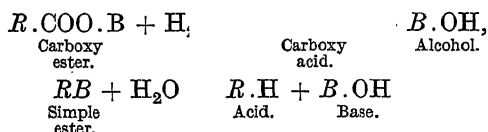
Enzymes causing resolution of the —C—C— bond.

1. ESTERASES

Representative Esterases

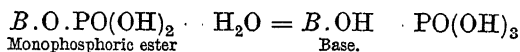
Name.	Source.	Substrate.	End-products.
Pancreatic lipase .	pancreas	fats and other	aliphatic acids and
Gastric lipase .	stomach	organic esters	glycerol.
Plant lipase .	seeds	" "	" "
Liver lipase .	liver	simple esters	acid " and alcohol.
Phosphatase .	yeast	phosphoric esters	phosphoric acid and
	cartilage		base
Sulphatase .	fungi	sulphuric esters	acid sulphates and
			phenols.
Tannase .	fungi	tannins	gallic acid and glu-
			cose.
Chlorophyllase .	green leaves	chlorophyll	chlorophyllin and
			phytol.

The esterases hydrolyse carboxylic and other esters in accordance with the equations :—



Lipases.—These esterases preferentially activate fats. The chief are pancreatic lipase and plant lipase. The latter occurs in all seeds rich in oil, its commonest source being the castor oil bean (*Ricinus communis*). Castor lipase is insoluble in water, and is extracted along with the lipide in an oily emulsion.

Phosphatases attack a variety of phosphoric esters, including glycerophosphates, hexosephosphates, and nucleotides. They are widely distributed in yeasts, moulds, and other sugar-fermenting organisms, and in mammalian tissue, chiefly intestinal mucosa, nervous tissue, bone, and ossifying cartilage. They liberate free phosphoric acid in accordance with the equation :—



Phosphocreatinase, which hydrolyses phosphocreatine, and *phospholecithinase*, which releases phosphate from lecithin, are examples of more specific phosphatases found in animal tissues.

2A. POLYSACCHARIDASES

Polysaccharidases attack polymerised carbohydrates, such as starch and cellulose, and convert them into sugars.

Polysaccharidases

Name.	Source.	Substrate.	End-products.
Amylase (diastase) {	pancreas	starch,	maltose.
Cellulase . . .	malt	glycogen	
Inulase . . .	fungi	cellulose	cellobiose.
	plants	inulin	fructose.

Amylase, or diastase, denotes a family of enzymes characterised by their power of depolymerising starch (*amylum*) and glycogen, with the production of a series of intermediate dextrins, leading eventually to maltose.

Polysaccharide.

Disaccharide.

Amylases are accompanied often by maltases, and, as a result, the end-product of the zymolysis is glucose, formed by the action of the maltase on the disaccharide. Amylases occur in the liver and digestive secretions of animals, in the roots, seeds, and germinating shoots of plants, and in many micro-organisms. The saliva of man the pig, the rat, and other mammals contains an amylase termed **ptyalin**; it is absent usually from the saliva of dogs and carnivora. The pancreas is the richest source of the enzyme in the animal. Industrially, amylase is obtained in quantity from extract of malt, or germinating barley.

Malt amylase has been resolved into a dextrinogen, α -amylase, which attacks central glucoside linkages in the starch molecule; and β -amylase which splits off maltose units step by step from the ends of the parent molecule.

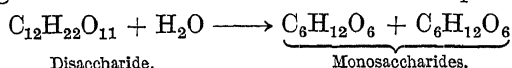
Cellulase is secreted by many bacteria and soil organisms, by wood-destroying fungi, and some marine worms. It is a necessary agent for all organisms that attack the natural cellulose of wood, leaf-mould, and timber. Cellulose is broken down into the disaccharide cellose (cellobiose), which, in turn, is converted into glucose by the enzyme *cellobiase* that often accompanies cellulase.

Cellulose is not found in high animals; and the celluloclastic powers of herbivora are ascribed to the presence of micro-organisms in their digestive tracts.

2B. SACCHARIDASES

Saccharidases attack compound sugars and convert them into simple sugars.

Three *trisaccharidases* are known (raffinase, melizitase, and stachyase), the rest of the group being *disaccharidases*, or enzymes hydrolysing disaccharides in accordance with the simple equation :—



Disaccharidases

Name.	Source.	Substrate.	End-products.
Maltase . . .	malt, intestinal secretions	maltose	glucose + glucose.
Sucrase, or saccharase (invertase).	yeast, intestinal secretions	sucrose	fructose + glucose.
Emulsin . . .	plants	glucosides	aglucone + glucose.
Lactase . . .	intestinal secretions	lactose	galactose + glucose.
Cellobiase . .	<i>Aspergillus</i> fungi	cellobiose	glucose + glucose.

Maltase accompanies amylase in animal tissues and completes the hydrolysis of polysaccharides. It is present in many bacteria, fungi, and yeasts, and in an insoluble form in ungerminated cereals. The optimal range of activity is about pH 6·1 (intestinal maltase) to pH 4·1 (malt-extract maltase).

Sucrase, saccharase or invertase is very widely distributed, occurring in the intestinal secretions of most animals, including the bee (as honey invertase) and the snail. It is found in all parts of plants, sugar beet being particularly rich, in fungi, bacteria, and yeasts (except true wine yeasts). All these preparations of the enzyme hydrolyse or “invert” cane sugar (sucrose) into “invert sugar,” an equimolecular mixture of fruit and grape sugar.

Lactase occurs in the intestine of all young mammals, although it may disappear in later life unless milk be part of the diet. It occurs in plants, especially almond seeds, *Aspergillus oryzae*, and the milk-sugar yeasts, but not in “top-fermentation” yeasts. Lactase converts lactose into galactose and glucosè at the optimal pH of 4·3 for almond lactase, and 7·0 for yeast lactase.

3A. PROTEINASES

Proteoclastic enzymes can be divided into two groups, the proteinases or *endopeptidases* which are capable of attacking central in preference to terminal peptide linkages; and *exopeptidases* (formerly termed peptidases or ereptases), which only attack terminal linkages. All enzymes which attack native proteins, albumins, globulins and their higher derivatives, split off many

different polypeptides from the large parent molecule, and for this reason are classified as endopeptidases. Both groups of enzymes are necessary for the complete hydrolysis of proteins.

Three chief types of proteinase occur: *Pepsinases*, acting in acid solution; *trypsinases*, acting in alkaline solution; and *papainases*, acting on proteins in neutral solution.

Representative Proteinases (Endopeptidases)

Name.	Source.	Substrate.	End-products.
Pepsin	stomach	proteins	peptones.
Trypsin	pancreas		"
Cathepsin	spleen, etc.	tissue " protein	"
Papain	melon tree	native protein	peptides.
Bromelin	pineapple	"	"
Rennin	stomach	caseinogen	casein.
Protaminase	intestine	protamines	peptides.

Pepsin.—The digestive power of gastric juice on meat protein was demonstrated by Spallanzani, about 1780, and the agent was located and named "pepsin" by Schwann in 1836. It is the only mammalian enzyme that acts in a strongly acid medium equal to $\frac{N}{10}$ HCl. At values less acid than pH 4 the activity of pepsin rapidly

approaches zero. Pepsin attacks all native proteins except the protamines and the scleroproteins. Zymolysis stops when the peptone stage is reached.

Trypsin.—The protease of pancreatic juice is secreted in an inactive form termed **trypsinogen**, and is activated by union with the co-enzyme enterokinase of the intestinal secretion. For this reason, the protease is sometimes called "trypsin-kinase."

Trypsin resembles pepsin in its power of attacking native proteins. In the pancreatic juice it is accompanied by various peptidases which act on the peptones produced by trypsin (and pepsin), and convert them into amino acids. The presence of these peptidases as contaminants was only established in 1929, it being formerly believed that trypsin could exert a peptoclastic property of its own.

Trypsin differs sharply from pepsin in its pH range. It works only in alkaline solutions of about pH 8–pH 11. The work of Bergmann shows, however, that the pH range of pepsin, papain and trypsin depends largely on the type of substrate attacked (1938).

Distinctions between Pepsin and Trypsin.—According to Hugounenq and Loiseleur (1926), pepsin retains its proteoclastic activity after its free $-\text{NH}_2$ groups have been methylated by

formaldehyde or destroyed by nitrous acid. Proteins treated in the same way are no longer attacked by pepsin.

Trypsin, on the other hand, is destroyed by methylation or deamination, although untreated trypsin is able to attack proteins that have been methylated or deaminated. From this, we conclude that pepsin functions by means of its carboxyl groups interacting with the free amino groups of the protein substrate; while trypsin functions by means of its amino groups interacting with the carboxyl groups of the substrate.

Rennin, Rennet, Chymase.—A phosphoproteinase found chiefly in gastric juice, but also present in the pancreas, and various other animal and plant tissues.

In the presence of calcium it converts soluble caseinogen into insoluble casein (paracasein), the optimal pH being 6.0–6.5. This causes the coagulation of milk.

Cathepsin.—The chief representative of the intracellular proteinases found in animal tissues, and responsible for autolytic digestion. Cathepsin belongs to the papainase type in that it attacks proteins in neutral solution, and is activated by thiol compounds (reduced glutathione, hydrogen sulphide) and by cyanide.

3B. PEPTIDASES (EXOPEPTIDASES)

These widely distributed enzymes differ from pepsin and purified trypsin by the fact that though unable to attack native proteins they act readily on peptides of all degrees of complexity, and resolve them into amino acids. **Erepsin** is an important mixture of peptidases found in the secretion of the intestinal mucosa. Similar mixtures are obtained from higher plants, yeasts, and micro-organisms. They can be resolved into peptidases displaying a high

Classification of Peptidases (Bergmann, 1937)

Endopeptidases (proteinases).	Exopeptidases.		
Split Central Peptide Linkages, R—CO—NH—R.	Split Terminal Linkages in Peptides.		Split Peptides with Proline Linkage, $\begin{array}{c} \text{—C} \\ \diagup \quad \diagdown \\ \text{—C} \quad \text{N—C.} \end{array}$
	With one Additional Active Group.	With several Additional Active Groups.	
Papainpeptidase Cathepsin Bromelain Pepsin Trypsin	Aminopeptidase Carboxy- peptidase	Dipeptidase Prolinase	Prolidase

degree of specificity for different peptides. In order to be susceptible to peptidase attack the peptide must contain at least one free amino or one free carboxyl group. *Dipeptidases* require both groups in their substrate.

Natural digestive secretion, such as gastric and pancreatic juice, gland and tissue extracts and unicellular organisms contain characteristic mixtures of proteases whereby they are able to resolve a variety of proteins into the constituent amino acids.

Proteoclastic Systems of Different Origin (Waldschmidt-Leitz, 1936)

Pancreas-intestine.	Glandular Tissue.	Yeast.	Leucocytes.
Pancreatic-proteinase (trypsin-type) Protaminase Amino-peptidase Carboxypeptidase Dipeptidase Prolinase Prolidase	Cathepsin (papain-type) Amino-peptidase Carboxy-peptidase Dipeptidase Prolinase	Proteinase (papain-type) Amino-peptidase Carboxy-peptidase Dipeptidase Prolinase	Proteinase (trypsin-type) Cathepsin Amino-peptidase Carboxy-peptidase Dipeptidase

4. AMINASES AND AMINE OXIDASES

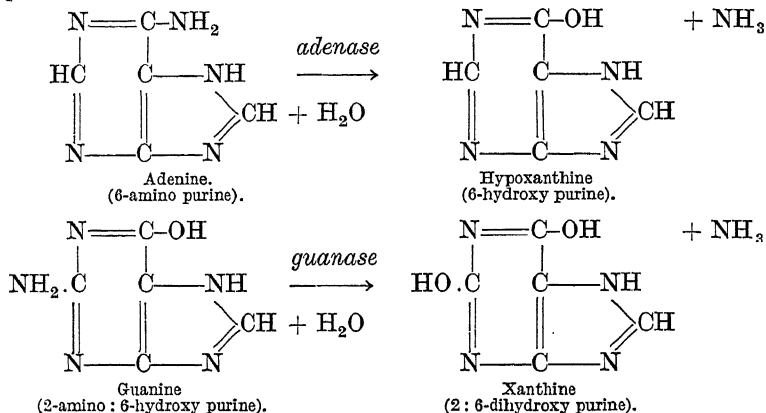
A miscellaneous group of enzymes exist that are capable of activating the linkage —C—NH— in non-protein compounds, nitrogen being liberated usually in the form of ammonia. Many of these enzymes are really members of the dehydrogenase family.

Representative Aminases

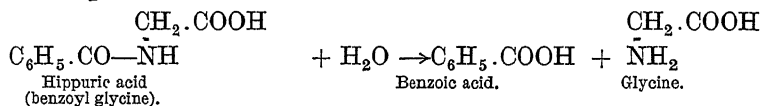
Name.	Source.	Substrate.	End-products.
Adenase .	liver	adenine	hypoxanthine.
Guanase .	liver	guanine	xanthine.
Allantoinase .	seeds	allantoin	allantoic acid.
Hippuricase .	kidney	hippuric acid	benzoic acid and glycine.
Arginase .	liver	arginine	ornithine and urea.
Urease .	Soy bean	urea	ammonia and carbon dioxide.
Asparaginase .	Aspergillus	asparagine	aspartic acid.
α -deaminase .	kidney	α -amino acids	keto acids
Glutaminase .	brain	glutamine	glutamic acid.

Adenase and **Guanase** deaminate the purine derivatives, *adenine* and *guanine*, respectively. Both enzymes are found in the liver and spleen of cattle and other mammals. Pig spleen is exceptional

in containing adenase only. The changes are important stages in purine metabolism, and are as follows:—

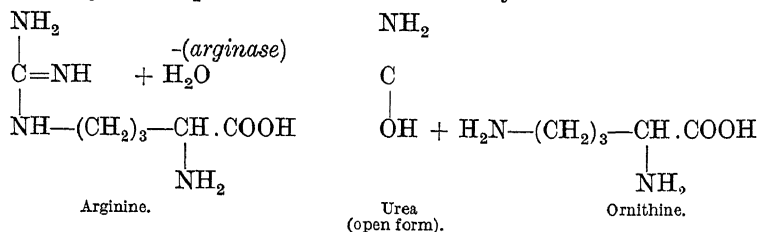


Hippuricase, or histozyme, acts on hippuric acid and its homologues. The enzyme occurs in the liver and also in the kidney, where it may bring about a reversion synthesis of hippuric acid from its components.



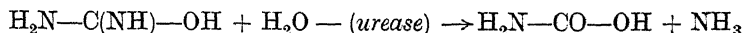
Arginase occurs in the livers of all animals that are *ureotelic* in that they excrete their waste nitrogen chiefly in the form of urea. It is absent from the livers of *uricotelic* animals (birds and most reptiles), which excrete nitrogen as uric acid. Traces of the enzyme may occur in non-hepatic tissue, and it has also been identified in plants.

Arginase specifically hydrolyses natural or (+)-arginine into the diamino acid ornithine and urea, and is part of the mechanism whereby urea is produced in the animal body.



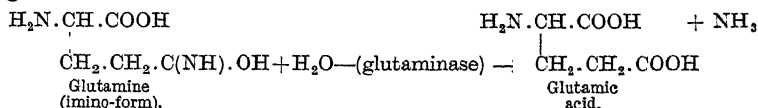
Urease, the enzyme responsible for the alkaline fermentation of urine, is widely distributed through the vegetable kingdom,

especially in root nodules and seeds of *Leguminosae*, important sources being the Soy bean, the Sword bean and the Jack bean, from the last of which urease has been isolated as a crystalline protein by Sumner. The enzyme, as might be expected, is absent from the tissues of higher animals, apart from an occasional appearance in gastric juice. Urease rapidly attacks urea, with formation of ammonia and carbamic acid, which breaks down into ammonia and carbon dioxide.



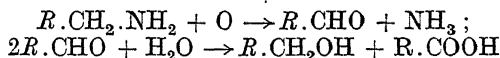
Urease is a specific enzyme, and its use provides an exact method for the detection and estimation of urea in blood and tissues.

Glutaminase occurs in brain cortex and retina of vertebrates, and in rabbit and guinea pig kidney. It converts glutamine into glutamic acid and ammonia.



AMINE OXIDASES

Biological deamination is usually accompanied by oxidation of the substrate, with the formation of an aldehyde, two molecules of which subsequently react to form alcohol and acid in accordance with the equations :

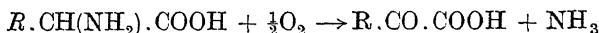


The first reaction is catalysed by the amine oxidase, the second reaction may take place spontaneously or under the activation of a separate enzyme, *aldehyde mutase*.

Many of the enzymes termed aminases are really amine oxidases, and confusion may be caused by the overlapping of the terminology, which is aggravated by the fact that amine oxidases are also included among the oxido-reductases in some classification.

Amine oxidase is widely distributed among vertebrate and invertebrate tissues, and attacks many aliphatic and aromatic amines, including adrenaline and tyramine. It is distinct from *histamine oxidase*, which oxidises diamines.

Amino acid oxidase, α -deaminase, converts α -amino acids into their corresponding keto acids :



The process is an aerobic deamination employing molecular oxygen, as denoted by $\frac{1}{2}\text{O}_2$.

The enzyme occurs in mammalian liver and kidney, from which sources Krebs (1935) has separated two types : L-deaminase, which attacks the natural amino acids, and which can be inhibited by octyl alcohol ; and D-deaminase, which oxidised the optically isomeric D-amino acids, and is not inhibited by octyl alcohol. Since these D-acids are foreign to living organism and not found in food proteins, the significance of their deaminase is obscure, unless it be to protect the animal from evolutionary freaks of metabolism and malignant growths (p. 136).

OXIDO-REDUCTASES

The oxidation of a substrate is always accompanied by the reduction of another compound, usually termed the oxygen-donor or hydrogen-acceptor. Consequently, the enzymes concerned in oxidation are grouped as oxido-reductases, since they may function either by activating the substrate so that it loses hydrogen, or activating the donor so that it loses oxygen. Oxido-reductases include :

- (1) Dehydrogenases, a type of highly specific enzymes represented in all living tissues, which bring about oxidations indirectly by transferring activated hydrogen from the substrate to a suitable acceptor. They differ from most of the other oxido-reductases in being inhibited by narcotics, such as urethane, but not by cyanide, carbon monoxide or hydrogen sulphide in low concentration. The principal dehydrogenases are described in connection with tissue respiration, in Chapter XIX.
- (2) Oxidases catalyse oxidations in presence of free oxygen which is simultaneously reduced to hydrogen peroxide.
- (3) Peroxidases transfer oxygen in active form from peroxides.
- (4) Catalase, though not an oxidising enzyme, is usually included with the oxido-reductases as it has the power of decomposing hydrogen peroxide into water and molecular oxygen.

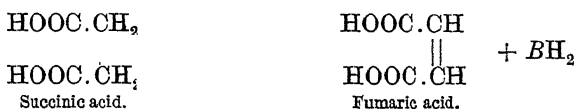
Representative Oxido-reductases

Enzyme.	Substrate.	End-product.
Glyoxalase.	Methyl glyoxal	Lactic acid.
Xanthine oxidase.	Hypoxanthine.	Uric acid.
	Xanthine.	Uric acid.
Aldehyde oxidase.	Aldehyde.	Acid.
Cytochrome oxidase.	Reduced cytochrome.	Oxidised cytochrome.
Diaphorase.	Reduced co-enzymes.	Oxidised co-enzymes.
Tyrosinase.	Tyrosine.	Melanin.
Carbolicase.	Pyruvic acid.	Acetylmethyl carbinol.
Lactic dehydrogenase.	Lactic acid.	Pyruvic acid.

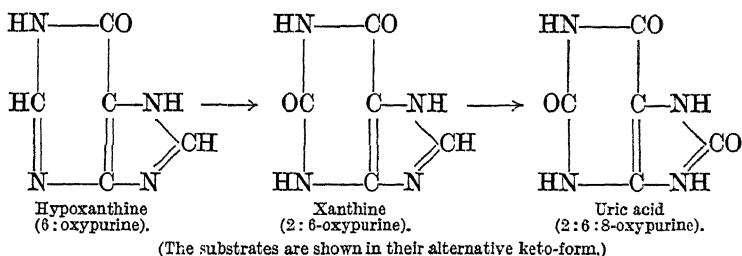
Glyoxalase, found in liver, yeast, and many tissues, converts methyl glyoxal to lactic acid, in presence of glutathione, its co-enzyme.



Succinic dehydrogenase is active in most animal tissues, and may be obtained by extracting previously-washed muscle tissue with alkaline phosphate solution. It catalyses the oxidation of succinic acid into fumaric acid in presence of atmospheric oxygen, or a **hydrogen acceptor**, such as methylene blue.



Xanthine oxidase oxidises the purines *hypoxanthine* and *xanthine*, and is concerned in the formation of uric acid :—

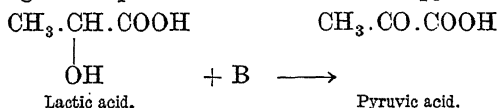


Aldehydes as a class are also oxidised by xanthine oxidase.

The enzyme is very irregularly distributed. It is completely absent from skeletal and cardiac muscle of all species examined. The liver is usually rich, except in the dog, hedgehog, and pigeon, from which it is absent. It is present in cow's milk, from which source it has been separated by Dixon.

Uricase, or *uric acid oxidase*, converts uric acid to allantoin (p. 346). The enzyme occurs in the livers of nearly all mammals, and in the kidneys of oxen, pigs, dogs, rats, and frogs.

Lactic dehydrogenase, in presence of methylene blue or other hydrogen acceptors, oxidises lactates to pyruvates.



It appears to be specific for α -hydroxy acids, converting them into the corresponding keto acid.

Lactic dehydrogenase occurs freely in animals and plants, its principle sources being yeast.

Oxidases catalyse oxidations in presence of free oxygen. Unlike the dehydrogenases, they are unable to use methylene blue, cytochrome or similar hydrogen acceptors, and they also differ in their sensitivity to inhibitors. Thus, urethane and related narcotics have no action on oxidases, but cyanide, carbon monoxide or hydrogen sulphide in concentrations as low as 0.01 M to 0.001 M inhibit them completely. This is ascribed to the fixation of an active metallic radicle, usually Fe, in the oxidase effector mechanism.

Cytochrome oxidase or **indophenol oxidase** is widely distributed in animal and plant tissues, especially those rich in nuclei, and usually accompanies cytochrome in distribution. It is absent from peripheral nerve tissue. The enzyme brings about the re-oxidation of cytochrome which has become reduced by accepting hydrogen from a substrate activated by a dehydrogenase (p. 333).

In 1885, Ehrlich showed that animals after injection of a mixture of *p*-phenylenediamine and α -naphthol, developed a blue colour in their tissues, owing to formation of an indophenol pigment. Batelli and Stern, in 1912, found that this property was common to almost all mammalian tissue, and ascribed it to the presence of an enzyme, indophenol oxidase. Keilin, in 1929, discovered the significance of the enzyme when he showed that it was able to re-oxidise cytochrome, and hence forms part of an important oxidation system in the living cell.

Tyrosinase occurs in lower animals, especially meal-worms, in many plants, notably clover, potato, and higher fungi. It catalyses the oxidation of the amino acid tyrosine with the production of a red pigment which turns black and becomes insoluble, in which condition it is called "melanin." Tyrosinase is believed to function in the formation of natural pigments from tyrosine, and also in the metabolism of the amino acid in lower organisms.

Onslow (1915) has shown that skin and hair pigmentation depends on melanin formation by tyrosinase. In *albinism* (recessive whiteness) there is a complete failure to inherit the enzyme; in dominant whiteness an enzyme inhibitor is present which prevents the formation of melanin.

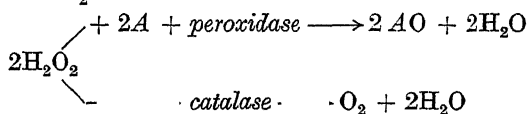
Peroxidases effect oxidations by causing hydrogen peroxide to transfer active oxygen to the substrate. Hæmatin derivatives and some other metallo-organic compounds are heat-stable peroxide catalysts, and are able to produce pigments from phenols and aromatic diamines (pyrogallol, guaiacol, benzidine, amidopyrine) in presence of hydrogen peroxide. This reaction is the basis of several tests for blood pigment. The position of peroxide in the living cell is obscure, as it is a powerful tissue poison. However, the majority of aerobic tissues contain both heat-labile peroxidases (enzymes) and heat-stable peroxide activators.

Mutase.—Dixon and Lutwak-Mann (1937) have shown that two distinct enzymes are capable of catalysing the oxidation of aldehydes: (i.) *Aldehyde oxidase* (Schardinger enzyme), which converts both aliphatic and aromatic aldehydes into the corresponding acid; and (ii.) *aldehyde mutase* (Parnas enzyme), which catalyses the Cannizzaro reaction, or mutation of two molecules of aldehyde into one of alcohol and one of acid.

Aldehyde oxidase occurs in milk, and requires no co-enzyme; aldehyde mutase is absent from milk, but well-represented in muscle and liver, and requires co-dehydrogenase I (co-enzyme I) for its effect.

Catalase.—A ubiquitous and important enzyme found in all aerobic tissues. It catalyses the decomposition of hydrogen peroxide into water and inactive, molecular oxygen, thus protecting the tissues from the effects of hydrogen peroxide produced during aerobic oxidations. Catalase has recently been isolated as a crystalline hæmo-protein from beef liver by Sumner (1937).

Peroxiase differs from catalase in that it only decomposes hydrogen peroxide in presence of an oxidisable substrate, or oxygen acceptor, *A*, and thus functions as an oxidising enzyme. Like catalase, it is a hæmatin derivative, and therefore inactivated by HCN, CO or H₂S.



Efficiency of the Enzyme Mechanism.—A highly active catalase has been prepared from horse liver by Zeile and Hellström, and shown to be an iron porphyrin with a spectrum resembling alkaline hæmatin. Haldane has calculated that at 0° C. one molecule of this enzyme catalyses the decomposition of about 2×10^5 H₂O₂ molecules per second.

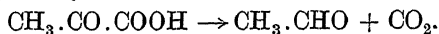
He observes that although enzymes in the cell are, in general, acting neither at their optimal temperature, pH, nor substrate concentration, it would seem that an enzyme molecule commonly must transform 100 or more molecules per second.

“As a very active cell, such as baker’s yeast, metabolises 10^{-6} of a gram molecule of O₂ per gram dry weight per second, we can get some idea of the possible complexity of the transformation undergone in it. If 5 per cent. of the dry weight of the cell consists of enzymes of an average molecular weight of 50,000, then 1 gm. dry weight contains 10^{-6} gm. molecule of such enzymes, and less than 1 per cent. of this would be required for any particular process, *e.g.*, the activation of O₂. In other words, the average atom on its metabolic path through the cell may be dealt with by more than 100 catalysts in succession.”

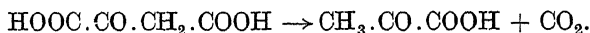
DESMOLASES

Desmolases attack the inter-carbon linkage, and thereby effect the cleavage of organic compounds during tissue respiration, muscle glycolysis, sugar fermentation, and similar events intimately associated with the continuous maintenance of life. Most of the desmolases are endo-enzymes, and occur as members of complex systems, accompanied by other enzymes, co-enzymes and activators.

Carboxylase, de-carboxylase, occurs freely in plants and animal tissues. It decarboxylates organic acids by liberating CO_2 from the $-\text{C}.\text{COOH}$ linkage. Vegetable carboxylase, such as occurs in yeast, only activates α -ketonic acids, converting them into the corresponding aldehyde.



Oxaloacetic carboxylase from animal tissue converts its substrate into pyruvic acid by a similar type of reaction :—



Carboxylases are responsible for the ultimate liberation of CO_2 in all biological oxidations. Their activity requires the presence of the co-enzyme, *co-carboxylase* or vitamin B_1 pyrophosphate.

Carbamic anhydrase, although not a desmolase, decarboxylates carbonic acid, and catalyses the converse hydration of carbon dioxide, thus controlling the rate at which the gas leaves the pulmonary circulation.

The enzyme, discovered by Meldrum and Roughton, is almost completely restricted to the red blood cells and the pancreas, where it may aid in the production of the alkaline pancreatic secretion.

It is a zymo-protein containing zinc (Keilin).

EXTRACTION AND PURIFICATION OF ENZYMES

No general method of enzyme preparation is available. Some, the desmo-enzymes, are restricted to the interior of cells ; others, the lyo-enzymes, escape into secretions and culture fluids. Purification requires removal of living organisms and inert cellular constituents, including proteins and other colloids likely to adsorb the enzyme. At the same time, the various co-enzymes and activators must be preserved, otherwise the activity of the enzyme will decrease during purification. Four methods are in use :—

(1) Fractional precipitation with alcohol All enzymes are insoluble in alcohol, and may be precipitated on addition of excess. The volume required depends on the nature of the enzyme and the impurities present. Thrombokinas is precipitated by 10–15 per cent. ; rennin requires 80–90 per cent. Acetone and other organic precipitants are used also.

(2) Fractional "salting-out," by addition of ammonium sulphate or similar salt. The proteins and other colloid impurities are removed in the first precipitation. Excess of salt is got rid of by dialysis of the final precipitate.

(3) Dialysis. This method is used to remove inorganic salts naturally present, or added during the process of purification. The enzyme is frequently inactivated during dialysis owing to loss of co-enzyme, or activators, or owing to adsorption on to the dialysing surface.

(4) Specific adsorption. Enzymes showing acidic properties can be removed from solution by addition of an insoluble basic adsorbent, usually aluminium hydroxide. Basic enzymes can be removed by use of an acidic adsorbent, usually kaolin. The adsorbed enzyme is liberated, or eluted, from the precipitate by changing the pH of the mixture or by addition of a more acceptable adsorbent.

Alumina adsorbs acid dyes, crude pancreatic lipase, pancreatic peptidase, plant peroxidase, and other acidic enzymes. Kaolin adsorbs basic dyes, trypsin, and other basic enzymes. Pancreatic amylase is not adsorbed by alumina or by kaolin, hence by the successive use of these adsorbents the three chief enzymes in pancreatic juice may be separated.

Chemical Nature of Enzymes.—Seven enzymes and two enzyme-precursors or *zymogens* have now been obtained in the form of crystalline proteins. They are: *urease*, *catalase*, *pepsin*, *trypsin*, *chymotrypsin* (the milk-coagulating enzyme of the pancreas); and the *zymogens* *trypsinogen* and *chymotrypsinogen* (Northrop, 1930-35); pancreatic *carboxy-peptidase*, and *papain*. Others obtained in conditions of approximate purity are: *sucrase*, *peroxidase*, *lipase*, *xanthine oxidase*, and *amylase*.

From variations in the composition of their preparations Willstätter and Waldschmidt-Leitz have been led to a *bearer* theory of enzyme structure, according to which each enzyme consists of (1) a colloidal bearer, usually of protein nature, and (2) one or more active groups which enable the bearer to become affixed to the substrate. The distinction between the soluble *lyo-enzymes* and the insoluble *desmo-enzymes* is determined by the solubility of the protein bearer. Sumner and Northrop, however, maintain that the crystalline enzymes are pure proteins, and that even partial hydrolysis of the protein destroys the enzyme. While, of course, it is possible that both theories may be true in that they apply to different enzyme types, the balance of evidence supports the conclusions of the American workers, and leads to the recognition of a new class of biochemical compounds, the *zymo-proteins*.

Mechanism of Enzyme Action.—Two processes occur in *zymolysis*: (a) adsorption of the substrate by the enzyme, (b) activation and transformation of the substrate while bound to the enzyme surface. That *zymolysis* is a surface or adsorption phenomenon is shown by the fact that the change does not proceed entirely in accordance

with the simple law of mass action (p. 208) in respect to both reactants.

By increasing the concentration of the enzyme, the rate of reaction is increased over a wide range, but when the concentration of enzyme is kept fixed while the concentration of the substrate is increased a point is reached when further addition of the substrate does not increase the rate of the reaction. When this point is reached, the enzyme surface is saturated with substrate, and remains saturated as long as excess of substrate remains in the mixture. Union between enzyme and substrate is determined by the presence of effector groups asymmetrically arranged in the "binding-plane" of the enzyme surface, and uniting with reciprocal groups in the binding plane of the substrate.

The pattern of these groups in space decides the specificity of the enzyme for a substrate or substrate type. Thus, many enzymes including hexases and peptidases have an antipodal specificity in that they will not attack the optical isomers of their substrates.

The substrate molecule must fit exactly into the mosaic pattern of the enzyme surface. The combination is only possible if enzyme and substrate can approximate in such a way that the two hypothetical binding-planes are not more than a few Ångström units apart (Bergmann, 1937). Thus, pancreatic peptidase will not attack synthetic peptides made from the non-natural D-amino acids.

The chemical nature of the effector groups depends on the substrate attacked. Dipeptidases and amino-peptidases combine with a free amino group in their substrates, possibly by means of an aldehyde group. The activity of papain, cathepsin, urease, and perhaps arginase, depends on their state of oxidation-reduction as determined by the $R-S-S-R + 2H \leftrightarrow R-SH + HS-R$ equilibrium of their effector groups.

Substrate Activation.—According to the "Two-affinities" theory of von Euler, the substrate is attached to the enzyme by more than one effector group and the resulting strain set up in the molecule leads to its rupture, when the fragments no longer held at two points escape into the body of the solution and are replaced by a fresh substrate molecule. Quastel has tabulated a number of organic groupings liable to undergo unstabilisation when in the enzyme surface field.

$R.CH : O$ $-OH$ activated aldehyde.

$R.CH = N$ $-NH.R$ activated imine derivative.

These activated substrates then combine with other reactants.

In this way Quastel has been able to explain and predict the behaviour of *Bacillus coli* towards 103 reagents, of which 56 were activated by the organism.

However activation be brought about, certain linkages appear to be specially susceptible to enzyme attack, as for example, the imino bond when in the neighbourhood of hydroxyl or amino groups.

Rates of Catalysed Reactions.—No simple general law is known that relates the three variables: enzyme concentration, substrate concentration, and rate of reaction under constant conditions of temperature and pH. Three cases may be recognised: (1) where the substrate concentration is so high that the enzyme is saturated during the greater part of the zymolysis; (2) where the substrate does not saturate all the enzyme; and (3) where the substrate concentration changes so much during the reaction that the degree of enzyme saturation falls from a maximum to a minimum.

The first is the simplest case. Assuming that the end-products do not inhibit the enzyme, the reaction is represented by $y = Vt$, where y is the amount of substrate transformed in the time t , and V is the velocity constant of the reaction. If the end-products inhibit the enzyme, as is usual, then V decreases with the time in a manner depending on the affinity of the particular enzyme, and expressed by various empirical equations.

Reversible Catalysis.—A catalysed reaction comes to rest either when all the substrate has been converted into end-products, or when the end-products have accumulated to such an extent that they are able to compete with the substrate for the surface of the enzyme. This is a typical condition of dynamic equilibrium.

The equilibrium level differs for different substrates. In ester systems, zymolysis usually ceases when about three-quarters of the original reactants are hydrolysed. In the dilute urea-urease system, zymolysis continues until about 99 per cent. of the urea is decomposed, and if the liberated ammonia be removed by aspiration the reaction continues to completion.

The same equilibrium level can be reached from either side of the equation. If the enzyme be added to a concentrated mixture of the end-products, **reversion synthesis** takes place with formation of sufficient of the original substrate to make an equilibrium mixture with the end-products. This reversion is of great biological importance, and is believed to be the mechanism whereby most animal and plant syntheses are brought about. Reversion is intracellular; the immobile enzymes are continually removing soluble metabolites and converting them into less soluble tissue constituents. For this reason, many enzymes and their natural substrates are found associated in nature. Thus, lipase occurs in seeds rich in oil, glucosides and glucosidases are present in plants, hippuricase accompanies hippuric acid in the kidney. This mechanism requires an additional factor to enable the reversion synthesis to continue

to build-up the substrate above the equilibrium level of the chemical system, otherwise the synthetic products could not accumulate to the extent normally found in tissues. Some agent is at work removing the surplus reversion products as they are formed. This may be an adsorption mechanism in the cell or some associated enzyme. Serial reactions are of common occurrence in biology, and it is probable that an individual reversion synthesis is only one rather obvious link in a long chain.

ACTIVATION OF PEROXIDES

(a) **Blood Pigments.**—(1) *Catalase effect*, liberation of molecular oxygen. Add 10 drops of 3 per cent. hydrogen peroxide to 5 ml. of blood diluted about 1 : 1,000. There is a rapid evolution of oxygen gas. Decomposition of the peroxide is effected by the successive reduction of the catalase iron by the peroxide, and its re-oxidation by the molecular oxygen (Keilin and Hartree, 1939), $4\text{Fe}^{+++} + 2\text{H}_2\text{O}_2 \rightarrow 4\text{Fe}^{++} + 4\text{H}^+ + 2\text{O}_2 \rightarrow 4\text{Fe}^{+++} + 2\text{H}_2\text{O} + \text{O}_2$

(2) *Peroxidase effect*, transfer of atomic oxygen to an oxygen acceptor (guaiacum). To 5 ml. of very dilute blood (1 : 5,000) add 5 drops of fresh, 2 per cent. alcoholic guaiacum resin and 5 drops of the hydrogen peroxide. Mix. A blue colour develops owing to oxidation of the guaiaconic acid in the resin to "guaiacum blue." Repeat the test, having previously boiled and cooled the dilute blood. The result is still positive, showing that the catalyst is heat-stable, and not an enzyme.

A slight opalescence forms when the guaiacum reagent is added to water, and must not be mistaken for a faint positive peroxidase reaction. This can be checked by use of a control tube.

Repeat the test, using (i) benzidine, and (ii) pyramidon as oxygen acceptors instead of guaiacum, as in the methods for detecting hæmaturia (p. 403).

Pure hæmoglobin has a powerful peroxidase effect, but very little catalase effect. Conversely, hæmatin, which arises in shed blood, has a powerful catalase, but only a feeble peroxidase effect (p. 227).

Non-metallic porphyrins do not display these catalytic properties.

(b) **Milk Peroxidase.**—Dilute 1 ml. of fresh milk with 5 ml. of water. Add 5 drops of guaiacum reagent and about 10 drops of turpentine that has been "activated" by exposure to moisture and light. Shake the mixture. A blue colour develops.

Previously boiled and cooled milk no longer gives the reaction, which is due to an enzyme and not a heat-stable catalyst.

Hydrogen peroxide may be used as the substrate instead of oxidised turpentine, but is liable to destroy the milk enzyme.

Some samples of milk give the blue colour on addition of guaiacum

alone, owing to the presence of traces of peroxide as well as peroxidase.

(c) **Potato Peroxidase.**—Repeat the experiments, using fresh potato scrapings, which are very rich in vegetable peroxidase, and also in catalase.

GENERAL REFERENCES

- BAILEY, K. C. (1937), "The Retardation of Chemical Reactions." London.
- BAYLISS, W. M. (1925), "The Nature of Enzyme Action," 5th Ed. London.
- BERGMANN, M., and J. S. FRUTON (1937), "On Proteolytic enzymes, XII." *J. Biol. Chem.*, **117**, 189.
- FALK, K. G. (1924), "The Chemistry of Enzyme Action," 2nd Ed. New York.
- HALDANE, J. B. S. (1930), "Enzymes." London.
- HOPKINS, F. G. (1931), "The Problems of Specificity in Biochemical Catalysis." Oxford.
- NORTROP, J. H. (1939), "Crystalline Enzymes." London.
- OFFENHEIMER, C., "Die Fermente und ihre Wirkungen." Suppl. 623 (1936). The Hague.
- RAPER, H. S. (1928), "The aerobic oxidases." *Physiol. Rev.*, **8**, 245.
- SCHOEN, M. (1928), "The Problem of Fermentation." London.
- SUMNER, J. B. (1935), "Enzymes." *Ann. Rev. Biochem.*, **4**, 37.
- TAUBER, H. (1937), "Enzyme Chemistry." London.
- WAKESMAN, S. A., and W. C. DAVIDSON (1926), "Enzymes." London.
- WALDSCHMIDT-LEITZ, E. (1929), "Enzymes." London.
- WALDSCHMIDT-LEITZ, E. (1931), "Mode of action and differentiation of proteolytic enzymes." *Physiol. Rev.*, **11**, 359.
- WALDSCHMIDT-LEITZ, E. (1936), "Recent advances in enzyme chemistry." *J. Soc. Chem. Ind.*, **55**, 620.

CHAPTER XIV

NUTRIENTS

“ ‘ All flesh is grass ’ is not only metaphorically but literally true ; for all those creatures we behold are but the herbs of the field, digested into flesh in them, or more remotely carnified in ourselves ; . . . this frame we look upon hath been upon our trenchers ; in brief, we have devoured ourselves.”

THOMAS BROWNE.

NUTRITION is the process whereby an organism incorporates material from its environment for the purposes of growth, maintenance and repair. A nutrient is a substance capable of being assimilated and utilised by an organism.

Foodstuffs are natural or manufactured products of plant or animal origin, used as sources of nutrients. Green plants and certain unicellular organisms are *autotrophic*, or self-supporting, and can grow and synthesise organic compounds when supplied with carbon dioxide, water, nitrate, phosphate and other inorganic solutes. Higher animals require carbon and nitrogen in complex forms, and are equipped with a specialised physiological system for dealing with their food materials.

In these organisms, the nutritional process involves acceptance, digestion, absorption, distribution and metabolism. Direct nutrients are assimilated unchanged ; indirect nutrients require chemical simplification previous to assimilation.

Classification of Food Materials

A. Chemical.		B. Biochemical.	
Inorganic	Organic	Direct	Indirect
1. Oxygen.	4. Carbohydrates.	1. Oxygen.	7. Poly- and com- pound saccha- rides.
2. Water.	5. Proteins.	2. Water.	8. Proteins.
3. Metallic salts.	6. Lipides.	3. Soluble salts.	9. Fats and lipoids.
	7. Micro-essential nutrients, vita- mins.	4. Simple saccha- rides.	
		5. Soaps.	
		6. Micro-essential nutrients, vita- mins.	

It will be seen that these classifications to some extent run parallel; the inorganic food materials, with the exception of insoluble salts, are assimilated without previous digestion, whereas most of the natural organic food materials require to be hydrolysed before they can pass from the alimentary tract to the portal system.

GROUP PROPERTIES OF THE FOOD MATERIALS

(1) **Oxygen.**—Oxygen is unique among foods. It is the only material accepted by the higher organism as a free element and in the gaseous form; it interacts with and liberates energy from the organic food materials; and it is not assimilated by means of the alimentary tract.

So important is the continuous provision of oxygen that a special apparatus, the respiratory system, has been developed for this purpose.

The adult human lungs have a volume of about 3 litres, and normally contain about $\frac{1}{2}$ litre of oxygen, which will suffice for the body at rest for about two minutes. If the supply is not maintained, a condition of anoxæmia develops rapidly, and death follows acute oxygen starvation in about three minutes.

(2) **Water.**—Water is not regarded popularly as a food material because it yields no energy to the organism. It is, however, the chief constituent of most of the tissues, and thus contributes to the structure of the body.

The total water intake of the normal adult is between 2–3.5 litres *per diem*, more than half of which is eaten in the form of solid food.

A 3,000-kilocalorie diet as consumed contains about 2,000 ml. of preformed and potential water and must be supplemented by the further consumption of 1 to 1.5 litres in a liquid form.

A satisfactory daily water intake for the adult is 1 ml. per kilocalorie of food.

Water Content of Prepared Foodstuffs Expressed in Percentage

Foodstuff.	Water.	Foodstuff.	Water.
Fruits	75–92	Eggs	73
Green vegetables . .	74–97	Cheese	23–60
Milk	80–95	Bread	24–40
Meat (cooked) . . .	48–77	Butter	11
Potatoes (boiled) . .	76	Biscuits.	5–10

The amount of water consumed has a minimal limit fixed by the osmotic tension of the circulating fluids. When the concentration

of certain solutes, such as NaCl, rises above the normal plasma level the sensation of thirst is evoked.

(3) **Metallic Salts.**—The biological metals are widely and unequally distributed in the dietary. Many of them occur as organic compounds, the nature and fate of which are obscure, but which are believed to undergo decomposition by the acid and alkali of the alimentary tract. Others occur as simple derivatives of chloride, carbonate, sulphate, and phosphate, the assimilable units are, presumably, the ions.

Dietary Sources of the Essential Biological Elements

Element.	Adult Requirement <i>per diem.</i>	Chief Sources.
Na	8–10 gm. as NaCl	Table salt. Animal foodstuffs.
K	1–3 gm.	Vegetable and animal foodstuffs.
Ca	0.7–1 gm.	Cheese, milk, cereals, egg-yolk.
Mg	0.2 gm.	All green vegetables, cereals, bran.
Fe	15 mg.	Liver, treacle, egg-yolk, lentils, parsley, cocoa, cereals, dried currants and raisins, lean meat.
Cu	3–5 mg.	Liver, cocoa, chocolate, oysters, mushrooms, treacle, nuts, cereals, egg-yolk.
Mn		Liver, kidney, pancreas, bran, tea, green vegetables, lean meat.
P	1–1.5 gm. as $\text{PO}_4^{''}$	Cheese, milk, egg-yolk, lean meat.
S	0.5–1 gm. as $-\text{SH}$	Egg albumin, milk, cereals, peas, beans and lentils.
Cl	8–10 gm. as NaCl	Table salt. Animal foodstuffs.
I	0.05 mg.	Crude table salt containing NaI, watercress, fish, liver and kidney.

(4) **Carbohydrates.**—About 60 to 80 per cent. of the solids of the human diet are carbohydrates, represented by four sugars (glucose, fructose, lactose, and sucrose) and three polysaccharides, glycogen, starch, and cellulose.

Glucose and fructose occur in syrups, honey, sweet fruits and preserves. Sucrose, obtained from sugar cane or sugar beet, is the common sweetening agent, although of late it is being supplemented by glucose (starch sugar) in the manufacture of preserves.

Lactose is obtained from milk, but, except in the diet of children, the amount consumed is small.

Glycogen is usually converted to maltose before it is consumed unless it be eaten in the form of raw oysters, fresh meat or liver.

Starch, the chief carbohydrate of the diet, occurs in bread, cereals, potatoes, root vegetables.

Cellulose, or vegetable fibre, does not contribute energy, since it is not attacked by any enzyme of the human alimentary tract. It may, however, have some service as a mechanical aid to peristalsis, and in

the form of bran and under the name of "roughage" it has prescribed, if not consumed, with enthusiasm.

Calorific Value.—Carbohydrates rank with the proteins as sources of energy value, the heat equivalent being about 4 kilocalories per gram.

Starch, being less hydrated, is 4.14; disaccharides are 3.9; and monosaccharides about 3.7.

Since 200–270 gm. of carbohydrate may be consumed in the ordinary daily dietary, it will be seen that it contributes about one-third of the total energy requirement of the adult. As a class, carbohydrates include the cheapest food materials, and for this reason they predominate in the dietaries of the poor and the parsimonious.

(5) **Proteins.**—The nitrogenous food materials make up 10–15 per cent. of the solids of the human diet, and may reach 30 per cent. if the consumer can afford them. They are the most expensive and most palatable part of the dietary, and are well represented in eggs, meat, fowl, fish, milk, cheese, nuts, cereals, and legumes (beans, peas, lentils). Proteins form the only dietary source of amino acids, the content of which differs in different proteins. For this reason, it is generally believed that about half of the food protein should be derived from animal sources to ensure getting a sufficient supply of the correct amino acids, and several sorts of protein are included in the dietary so as to provide an adequate selection.

Calorific Value.—When completely combusted into carbon dioxide, nitric acid, and water, 1 gm. of dry protein yields 5.8 kilocalories of energy. In the organism, however, oxidation of the nitrogen is only carried as far as urea, and for this reason the calorific value of food protein is only 4.1 kilocalories per gram.

The higher animal is an extravagant apparatus. It has evolved unequally, and has lost the power of synthesising many of the organic compounds necessary for its existence, and must depend on its dietary for these exogenic factors. In consequence, its food supply is varied and complex, and requires a series of digestive juices.

It transports its oxygen by a respiratory pigment that needs frequent renewing. During renal excretion of waste solutes it loses valuable compounds, such as vitamins and amino acids. And, perhaps most serious of all, its alimentary tract is heavily and continually infected by organisms, which demands the maintenance of a detoxication mechanism. If the animal is to be preserved

from destruction it must be provided with its calories in the form of *adequate* nutrients.

The Protein Requirements of Man.—The normal requirement for the human subject is rated at 1 gm. protein per kg. body weight, *per diem*. This allowance is made on the assumption that the protein of the diet is mixed and of a good quality. The protein intake must provide a sufficiency of the essential amino acids, of which at least five species are necessary : tyrosine or phenylalanine, tryptophane, cystine or methionine, lysine and threonine. Digestibility must also be taken into account, and it is now customary to assess proteins in terms of their *biological value* to the consumer. This is computed by a balance sheet method (Thomas, 1909 ; Chick, 1935).

The *biological value* of a protein X is expressed as :—

$$100 \times \frac{\text{Body nitrogen saved}}{\text{Food nitrogen absorbed}} = 100 \times \left\{ 1 - \frac{UN_x - UN_e}{IN_x - (FN_x - FN_e)} \right\},$$

where UN_x = Urinary Nitrogen excreted on a diet containing X ,

UN_e = Urinary Nitrogen excreted on a nitrogen-free diet,

IN_x = Food Nitrogen intake in the form of X ,

FN_x = Faecal Nitrogen excreted on a diet containing X ,

FN_e = Faecal Nitrogen excreted on a nitrogen-free diet.

UN_e and FN_e represent the endogenous nitrogen output, which is assumed to be constant irrespective of the diet. The maximum biological value of a protein on this scale is 100, which indicates the utilisation of all the absorbed food nitrogen.

With the exception of the milk proteins, the biological value of which remains high at all levels, increasing the proportion of protein in the diet from 3 to 5, 7 or 10 per cent. lowers the biological value.

Biological Value of Proteins (Chick *et al.*, 1935)

Protein	Low Level Intake.		High Level Intake.	
	Dietary percentage.	Biological Value.	Dietary percentage.	Biological Value.
Wheat, entire .	3.6	100	5.6	68
Wheat, germ. .	3.6	90	6.8	69
White flour .	3.1	84	7.0	61
Milk, dried .	3.4	89	6.9	81
Lactalbumin .	3.1	92	6.7	65

The average daily protein intake of 80–100 gms. provides about 400 kilocalories of energy, leaving a balance of some 2,600 kilocalories to be supplied by the lipides and the carbohydrates.

(6) **Lipides.**—These include the true fats and the lipines, although the latter are usually neglected in nutritional calculations. The true fats make up about 20–30 per cent. of the diet, depending on the temperature and season of the year.

Their chief sources are : animal fats (lard, suet, butter, cream), vegetable fats (oils, oleo-margarines, nuts). Fats are important as concentrated sources of energy, and represent a form in which energy is stored in animals and in the seeds of plants.

Calorific Value.—On account of their low oxygen content, fats have the high energy value of 9.3 kilocalories per gram.

The Lipide Requirements.—The lipides of the dietary differ from the other foodstuffs in that they carry as solutes various highly important micro-essential nutrients, including vitamins A and D and the provitamin carotinoids and sterols. The complex lipides also provide choline, the anti-liposis factor. The average consumption of lipide in temperate countries is of the order of 1 gm. per kg. body weight, *per diem*, and is ingested both as animal and vegetable fats. This intake varies with circumstances and seasons, but provides about 600 to 700 kilocalories *per diem*. Fat for its complete metabolism requires to be balanced by carbohydrate, and if the fat-content of the dietary exceeds a limiting value a ketosis occurs, due to the accumulation of aceto-acetic acid, and related products of incomplete fat combustion (p. 319). This ketogenic state is sometimes deliberately induced in the treatment of epilepsy and infections of the urinary tract. It cannot arise under dietary conditions where twice the total carbohydrate intake together with half the protein intake are greater in weight than the total fat intake.

VITAMINS

Vitamins are organic micro-constituents of the diet, and are necessary for the growth and maintenance of animals. They may be regarded as *exogenous* hormones which the organism is unable to manufacture for itself ; and like the autacoids or *endogenous* hormones of animal origin, they are therapeutic agents of great potency as well as determinants of normal growth and activity. Vitamins are classified provisionally according to solubility and chief therapeutic effect.

The Vitamin Theory.—The acceptance of the vitamins as an independent class of food constituents is due to the accumulation of evidence from five distinct sources : (1) the clinical recognition of rickets, scurvy, and beri-beri as deficiency diseases ; (2) the empirical use of natural products rich in vitamins, such as liver oils, lemon

I. *Fat-soluble Vitamins*

Name.	Formula.	Nature.	Action.
Vitamin A ₁ .	C ₂₆ H ₂₉ .OH	semi-β-carotene.	{ anti-keratinising. anti-xerophthalmic. anti-rachitic. calcio-kinetic.
Vitamin A ₂ .	C ₂₁ H ₃₀ .OH		
Vitamin D ₁ .	C ₂₇ H ₄₁ .OH (?)	natural steroid.	
Vitamin D ₂ . (calciferol).	C ₂₈ H ₄₃ .OH	irradiation product.	" "
Vitamin D ₃ .	C ₂₇ H ₄₃ .OH	natural steroid.	" "
Vitamin E . (α-tocopherol).	C ₂₉ H ₅₀ O ₂	chromane.	anti-sterility factor.
Vitamin F .	C ₁₈ H ₃₂ O ₂	linoleic acid.	growth factor.
Vitamin K ₁ .	C ₃₂ H ₄₈ O ₂	naphthoquinone	anti-hæmorrhagic.

II. *Water-soluble Vitamins*

Vitamin B ₁ . (thiamine).	C ₁₂ H ₁₇ ON ₄ SCl	thiazo-pyrimidine.	anti-neuritic.
Vitamin B ₂ (G)		D-riboflavin.	growth factor.
Vitamin B ₆ . (adermine).		pyridine	anti-dermatitis.
P.p. factor .	C ₆ H ₆ O ₂ N	nicotinic amide.	anti-pellagra.
Vitamin C .	C ₆ H ₈ O ₆	L-ascorbic acid.	anti-scorbutic.
Vitamin P . (eriodictyol, hesperidin).		flavone glucoside.	capillary factor.

juice, and yeast ; (3) the experimental proof that animals are unable to live and grow on dietaries of purified protein, carbohydrate, lipide, and inorganic salts ; (4) the production and cure of typical deficiency diseases ; (5) the separation and synthesis of the actual vitamins.

Historical.—In 1873, Forster, Voit's assistant in Munich, examined the effect of a de-mineralised diet on dogs and pigeons. Using purified protein, fat, and starch, he found that the animals died within a month, and concluded that " food deprived of its inorganic salts causes death more rapidly than total deprivation of food."

In 1881, Bunge repeated these experiments on mice in order to find which salts were necessary for life. He concluded that an additional factor was required, " although animals can live on milk alone, yet if all the constituents of milk which according to the present teaching of physiology are necessary for the maintenance of the organism be mixed together, the animals rapidly die . . . does milk contain in addition to proteid, fat, and carbohydrates, other organic substances which are also indispensable to the maintenance of life ? " (1902). Hopkins, in 1906, recognised the association between these unknown food factors and the widespread occurrence of deficiency diseases : " Scurvy and rickets are conditions so severe that they force themselves on our attention ; but many other nutritive errors affect the health of

individuals to a degree most important to themselves, and some of them depend on unsuspected dietetic factors."

A year later, Fraser and Stanton obtained by the alcoholic extraction of rice bran a product capable of curing beri-beri, and, in 1911, Funk obtained an active, crystalline residue; and, since it contained basic nitrogen, named it vitamine.

In an important paper, published in 1912, Hopkins showed the presence of "accessory food factors" in milk which were essential for the growth of rats. In 1915, these were resolved by McCollum and Davis into a fat-soluble A factor and a water-soluble B factor, and with them a third, the water-soluble C factor, was included by Harden and Zilva, in 1919. Since then the original A factor has been resolved into A and D by E. Mellanby, and the original B factor has been shown to be a mixture of several vitamins.

The potency of the vitamins is such that, with the exception of vitamin C, a daily dosage of the order of 0.01–0.1 mg. is adequate. They can contribute nothing appreciable to the energy of the organism or to the grosser composition of the tissues, and for this reason have been termed by Bertrand "catalysts of nutrition."

THE FAT-SOLUBLE VITAMINS

Then the Angel said to him : Take out the entrails of this fish, and lay up his heart, and his gall, and his liver, for thee : for these are necessary for useful medicines.

[Tobit, VI, 5.]

(1) **Vitamin A.**—This growth-promoting lipide vitamin was discovered independently by McCollum and Davis (1913) and by Osborne and Mendel (1913). It is known variously as fat-soluble A, growth-promoter A, and the anti-xerophthalmic factor.

Sources.—Vitamin A occurs chiefly in storage fats of liver, such as fish-liver oils, and in the fat of milk, cream, and butter. It or its precursors are found in egg-yolk, seed embryos, and, universally, in green, leafy vegetables, flowers, and fruits containing the yellow carotene pigments.

The vitamin is synthesised exclusively by plants, and is stored in animal lipide tissues. It is minimal in root vegetables and vegetable fats, such as olive oil, palm oil, coconut oil, and is absent from their derivatives—the hardened fats and oleo-margarines.

Properties.—Vitamin A, $C_{20}H_{29}.OH$, is an alcohol derived from semi- β -carotene, and may be replaced in nutrition by any one of the four carotenes containing the semi- β -carotene residue (α -carotene, β -carotene, γ -carotene, and cryptoxanthin). The formulæ of these compounds and vitamin A are given on pp. 202–3.

Transformation of the provitamins into the vitamin takes place in the liver, where the vitamin subsequently is stored. Vitamin A

is sparingly soluble in water, but dissolves freely in fats and fat-solvents, the solutions showing the characteristic absorption-band in the ultra-violet region at $328\text{ m}\mu$, also displayed by the vitamin itself, which when pure is a colourless oil. The vitamin is stable to heat, acids and alkalis, but is easily inactivated by reduction, and is destroyed during the hydrogenation of oils to form solid food fats. It is liable to be inactivated also, by the presence of vitamins of the B group and by vitamin C, which is a powerful reducing agent, and for this reason should not be administered therapeutically in concentrated mixtures.

Functions.—Vitamin A is necessary for the normal growth of young animals, and for the maintenance of adult tissues of epiblastic origin (central nervous system, retina, skin and dermal glands, oral and nasopharyngeal mucosa, salivary glands). No function has been ascribed to the vitamin in plant physiology.

Effects of Vitamin A Deficiency.—(1) *Failure of Growth.*—This is obvious only in young animals. The growth-rate falls off rapidly when the vitamin is withheld and the tissue-reserves have been exhausted. The animal continues to exist at a subnormal weight until secondary disturbances or infections develop.

(2) *Keratinisation of Epithelial Tissue.*—The most characteristic sign of avitaminosis A is the change seen in the skin, mucous membranes and conjunctiva. Dryness of the skin is followed by a papular eruption due to lack of secretion by the sebaceous glands of the hair follicles. This state of *phrynoderma*, or “toad skin,” has been recognised in Africa and in Asia as an early form of vitamin A deficiency in human subjects.

- (i.) *Xerophthalmia*, or keratosis and ulceration of the cornea. The lachrymal secretion is diminished, and the conjunctiva becomes dry and inflamed. Secondary infections lead to ulcerations, which, by involving the anterior chamber, may terminate in total blindness.

While the infection is independent of the vitamin, the predisposing syndrome is characteristic of avitaminosis A, and in the early stages yields specifically to treatment.

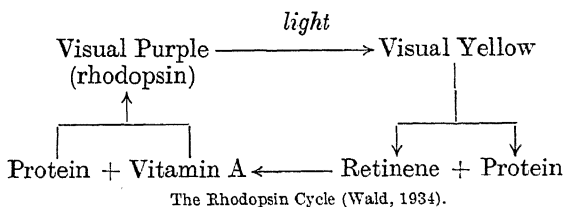
- (ii.) *Gastro-enteritis.*—The mucosa of the alimentary tract degenerates and the villi become necrosed. Ulceration of the stomach and intestines may set in subsequent to oral infection.
- (iii.) *Pharyngitis.*—Keratinisation of the naso-pharyngeal epithelium predisposing to inflammation of the upper respiratory tract.
- (iv.) *Nerve Degeneration.*—Mellanby (1934) claims that vitamin A is necessary for the maintenance of the nervous tissues,

and that avitaminosis A is responsible for the degenerative changes seen in beri-beri, pellagra, disseminated sclerosis of the cord, lathyrism, convulsive ergotism and pernicious anæmia. The principal evidence is the beneficial effect of vitamin A therapy in the treatment of disseminated sclerosis. But Harris observes (1936): "If chronic vitamin A deficiency is the cause of the nervous lesions . . . one might expect to see evidence of its better known symptoms, such as local infections and xerophthalmia."

- (v.) *Night-blindness*, or *Nyctalopia* (hemeralopia).—Before marked clinical signs appear, an early deficiency condition can often be inferred from ocular changes leading to loss of vision in dim light, and terminating in degeneration of the retina and optic nerve. The condition is common in Greece and in Newfoundland (Aykroyd, 1936), where it has been treated, empirically, for many years by fish-liver, a prescription adopted successfully by Tobit some three thousand years ago.

Avitaminosis A manifests itself collectively as a lowered resistance to infections, and for this reason the vitamin has been called "the anti-infective factor." The term is misleading, as the vitamin has not been found to have any curative effect in actual infections, but owes its importance to its histo-chemical position as a tissue component.

Vitamin A and Retinal Metabolism.—The rods of the retina contain a chromoprotein, *rhodopsin*, or visual purple, which is photo-sensitive, and participates in the conversion of light energy into transmissible nervous stimuli. Light changes rhodopsin into another pigment, *visual yellow*, which is subsequently decomposed into protein and a carotinoid, *retinene*. Wald, the discoverer of the reaction, has shown that retinene is converted into vitamin A, which recombines with the protein to regenerate rhodopsin, thus forming a photo-chemical cycle in the retina.



Vitamin A Standard and Requirements.—The international standard adopted by the League of Nations (1934) for estimating

and administering vitamin A is the biological activity of 0.6 γ of pure β -carotene, in coconut oil, stabilised by hydroquinone (1,000 γ = 1 mg.).

The nutritional standard in common use in America is one devised by Sherman, and represents the amount of vitamin or provitamin capable of maintaining a gain of 3 gm. a week in the weight of a young rat on a controlled diet. One Sherman A unit equals approximately 1.4 international units. The minimal daily requirement of vitamin A for the human adult, according to Fraps and Treichler (1933), is 1,000 Sherman units, but many workers prefer to exceed this recommendation, especially in child nutrition, and advise an intake of 2,000 international units, or the equivalent of about 3–6 mg. carotene or vitamin A, *per diem*. The most potent sources are pure carotene, halibut-liver oil (which has an actual vitamin content ranging from 0.1 to nearly 10 per cent.), salmon-liver oil and high-grade cod-liver oil. Satisfactory dietary sources are found in butter, cream, cheese, egg-yolk, liver, apricots, carrots, green lettuce, peas, spinach and watercress.

Representative Vitamin A Value of Common Foodstuffs
(Coward and Morgan, 1935)

Material.	Vitamin A in International Units.	
Milk . . .	3 units per ml.	1,700 units per pint.
Jersey . .	5 units per ml.	2,850 units per pint.
Butter . .	26–200 units per gm. (average, 60).	730–5,000 units per oz. (average, 1,700).
Egg-yolk .	30 units per gm.	60 units in a $\frac{3}{4}$ -oz. yolk.
Bone marrow	8 units per gm.	220 units per oz.
Carrots, fresh or boiled.	19 units per gm.	2,000 units per $\frac{1}{4}$ lb. portion.
Cabbage, fresh or boiled.	9 units per gm.	1,000 units per $\frac{1}{4}$ lb. portion.
Liver oil, cod	600–4,000 units per gm. (average, 2,000).	2,000–13,000 units per tea- spoonful (average, 6,400).
Liver oil, hali- but.	30,000–300,000 units per gm. (average, 160,000).	600–7,200 units per drop (average, 3,200).

As a guide, the chlorophyll content of plants is roughly proportional to the carotene value; and for this reason the green outer leaves of lettuce and cabbage are better foodstuffs than the pale inner leaves.

The vitamin content of animal foodstuffs is primarily determined by the carotene content of the animal's diet. Milk contains both carotene and vitamin A, depending on the species of animal and the time of the year.

Tests for Vitamin A.—In addition to the biological tests by feeding, the vitamin may be detected and estimated (1) *spectroscopically*, by observing the absorption coefficient at 328 m μ in the solution; and (2) *chemically*, by the antimony trichloride colour reaction (p. 204); (3) by measuring the time required for dark-adaptation in order that the subject may recognise a dimly-lit diagram.

Vitamin A₂ occurs in the liver and retina of fresh-water fish.

Carotene Values of Foodstuffs not Containing Vitamin A
(Fixsen and Roscoe, 1938)

Material.	Carotene in γ per 100 gm.
Cereals :	
Maize	10-900
Rice, milled	0
Wheat, whole grain or flour	102-456
bran	235-456
white flour	81-282
Fruits :	
Apricot, fresh	1,800-2,300
sun-dried	5,300
Date, preserved	600
Grape	15
Lime, juice	26
peel	305
Orange, juice	300-400 per 100 ml.
Vegetables :	
Beans, French	221-400
Beetroot	0
Cabbage, dry weight	16,400
Carrot, red	2,000-9,600
Celery, leaves	3,600
Lettuce	1,500-2,400
Pea	139
Potato	28-56
Spinach	5,600-6,500
Tomato, pulp	14,160-35,640
skin	265,200-886,200
Forage :	
Grass, pasture, dry weight	42,200-56,200
Hay, pasture	18-620
Miscellaneous :	
Tea, black, dry weight	71,500
Yeast, brewer's	110

It differs from vitamin A in its ultra-violet spectrum, and in the colour it gives with SbCl_3 . Otherwise, both vitamins are closely related, and may occur together in liver oils.

(2) **Vitamin D.**—The concept of an anti-rachitic vitamin is due to Hopkins, who, in 1906, suggested that rickets (or rachitis) is a nutritional deficiency disease. The anti-rachitic factor was believed to be vitamin A, but, in 1922, McCollum and his colleagues found that cod-liver oil, freed from vitamin A by aeration at 120°C ., still was effective in the cure of rickets. This they correctly attributed to the presence of a factor recognised in 1919 by E. Mellanby, as vitamin D.

In June, 1924, it was announced by Hess, and in September of the same year by Steenbock, that inert foodstuffs can be rendered anti-rachitic by ultra-violet irradiation. Two years later it was shown that a vitamin precursor occurred in the sterol fraction of the food material.

It was thought at first that *calciferol*, the synthetic vitamin obtained from ergosterol, was indetical with the natural vitamin D, but Bills showed that calciferol was less potent, and, subsequently, a third form of the anti-rachitic vitamin was obtained from dehydrocholesterol, a naturally occurring sterol, and it seems probable that other forms exist. In current nomenclature, vitamin D_1 is the natural vitamin present in liver oils; vitamin D_2 is calciferol, the irradiation product from ergosterol; and vitamin D_3 is the vitamin obtained by the irradiation or chemical activation of dehydrocholesterol. A fourth form of the vitamin, D_4 , has been obtained artificially by irradiation of 22, 23-dehydroergosterol (Windaus, 1937).

Sources.—Vitamin D is formed by the action of ultra-violet irradiation on sterols, and, consequently, it occurs in Nature wherever this reaction has taken place, the amount depending on the sterol content of the food material and the degree of exposure to sunlight or other source of radiant energy. Vitamin D is associated usually with vitamin A in distribution, and occurs freely in liver oils and other animal fats, including egg-yolk and butter. It is found in the leafy parts of most plants, but is absent from vegetable oils. The provitamin, ergosterol, occurs chiefly in yeast and higher fungi; material containing it is a potential source of vitamin D_2 , and may be rendered active by natural or artificial irradiation.

Synthesis of Calciferol.—Ergosterol is exposed to ultra-violet irradiation until the spectrum shows maximal absorption in the region 265–270 $\text{m}\mu$. The resulting mixture is fractionated, either by digitonin, which forms an insoluble addition compound with the unattacked ergosterol, or by distillation in high vacuo, which

removes the irradiation products. After subsequent purification, the vitamin can be recrystallised from methyl alcohol.

Properties.—Vitamin D₂, or calciferol, C₂₈H₄₃.OH, is isomeric with ergosterol, but differs in optical activity, ultra-violet spectrum, and other properties. The vitamin occurs in colourless needles, m.p. 114.5–117° C., $[\alpha]_D^{20} = +102.5^\circ$, in alcohol. The solutions show an intense absorption band in the region 265 mμ. The vitamin gives none of the carotinoid reactions characteristic of vitamin A.

Effects of Vitamin D Deficiency.—The vitamin regulates the absolute and relative absorption of calcium and phosphate solutes from the intestinal tract, and may be regarded as a *calcio-kinetic*, or calcium-distributing, vitamin. Avitaminosis D is characterised by:—

(1) *Subnormal phosphate level in the plasma.*—In man, the inorganic phosphate value of 5 mg. per 100 ml. may be lowered to 1.5 mg.

(2) *Subnormal calcium level in the plasma.*—The total calcium value may fall from its normal level of about 10 mg. per 100 ml. to half this amount.

Values below 7 mg. tend to induce hypocalcæmic tetany unless the fall has been so gradual that the organism has become adapted to the lower level.

(3) *Decrease in the Ca × PO₄ product in plasma.*—Rickets cannot occur if the product Ca (in mg. per 100 ml. plasma) × PO₄ (in mg. per 100 ml.) is above 40. Severity and rapidity of onset depend on the extent to which the product falls below 40.

(4) *Decrease in the excretion of phosphate and calcium by the kidney.*

(5) *Increase in the excretion of phosphate and calcium by the intestine.*—These conditions are ascribed to defective absorption from the alimentary tract, the contents of which tend to become abnormally alkaline in the absence of vitamin D.

Pathological Conditions Associated with Vitamin D Deficiency

(i) *Rickets.*—This disease, characterised by defective ossification of the growing skeleton, is determined by five ætiological factors: (1) the calcium content of the dietary; (2) the phosphate content of the dietary, including the phosphoproteins; (3) the supply of vitamin D; (4) the degree of exposure to ultra-violet irradiation; (5) the de-calcifying agents in the dietary.

The de-calcifiers are imperfectly understood. One, termed *calcio-varin*, has been found by Mellanby in oatmeal, and accounts for the severe form of rickets that follows excessive consumption of cereals. It may be neutralised by increased administration of vitamin D.

(ii.) *Osteomalacia.*—A disease rare in Europe but common among women in certain parts of China. It is an adult form of rickets, and is

characterised by de-calcification of the skeleton developing during the later months of pregnancy, when the resources of the mother are being exhausted to supply the foetus.

The domestic proverb, "Every child costs a tooth," is a recognition of this super-tax imposed on maternity.

According to Bruce and Callow (1934), cereals favour the production of rickets because most of their phosphorus is in the non-assimilable form of phytin, and their continued use leads to a phosphate starvation.

(iii.) *Dental Caries*.—M. Mellanby (1934) concludes that vitamin D, along with assimilable calcium and phosphate, is necessary for the formation of secondary dentine, and the defence against caries. Although the "average" tooth is of poor structure and liable to decay, its architecture may be improved by proper diet. She advocates a decreased consumption of cereals, or even their complete removal from the dietary of young children. Vitamin C also appears to be concerned in the maintenance of dental structure.

Vitamin D Standard and Requirements.—The international standard adopted by the League of Nations (1931) is the biological activity of 1 mg. of a standard solution of calciferol in oil. This quantity given daily to a young rat rendered rachitic by a diet free from vitamin D will produce in eight days a characteristic band of calcium deposits in the metaphyses of the long bones. One gm. of crystalline calciferol is equivalent to more than 40,000 international units. The daily requirements of vitamin D needed to prevent or cure mild rickets in children is about 3,000 units, in the form of calciferol, or 1,000 units in the form of liver oil (vitamin D₁).

Sources of Vitamin D.—Vitamin D, like vitamin A, is stored freely in the liver fats of fishes, the content varying seasonally, and inversely with the oil content. Expressed in international units per gm. liver oil, representative values are: bluefin tuna, 40,000; swordfish, 10,000; halibut, 2,100; turbot, 260; cod, 100; haddock, 10. The vitamin is also obtainable in egg-yolk, irradiated milk, yeast and other foodstuffs rich in sterols, as well as in the synthetic form, calciferol. Plant products as a class are devoid of vitamin D. An intrinsic source of the vitamin is provided by the direct insolation or artificial irradiation of the human subject.

Hypervitaminosis D.—Vitamin D is the only known vitamin capable of causing pathological disturbances when given in very large amounts. These disturbances include increased calcification of bone and abnormal calcification of soft tissues; the immediate cause, according to Harris, is the hypercalcaemia and hyper-

phosphatæmia produced by the vitamin. Hypervitaminosis D is improbable in human nutrition, the quantities of the vitamin ingested in the diet, or administered therapeutically are far below the danger level.

Representative Vitamin D Value of Common Foodstuffs
(Coward and Morgan, 1935)

Material.	Vitamin D in International Units.	
Milk . . .	0.0-0.1 unit per ml.	Not greater than 50 units per pint.
Butter . . .	0.4-4 units per gm. (average, 1.2).	10-100 units per oz. (average, 34).
Cream . . .	0.5 units per gm.	80 units per gill.
Egg-yolk . .	1.5-5 units per gm.	30-100 units per $\frac{3}{4}$ -oz. yolk.
Liver oil, cod	60-300 units per gm. (average, 150).	190-1,000 units per tea-spoonful (average, 480).
Liver oil, hali- but . . .	2,000-4,000 units per gm. (average, 2,400).	40-80 units per drop (average, 48).
Olive oil . .	Nil, even up to 20 per cent. of the diet.	

Vitamin D deficiency is a serious menace to children living in northern latitudes, and especially dwellers in crowded areas, where the sunlight available in the winter months is insufficient to synthesise the vitamin from body sterols.

Tests for Vitamin D.—*Biological* tests include calcification of the bones of young animals as shown by (i.) radiography of the distal ends of the ulna and radius; by (ii.) the "line test," in which the bone is sectioned and stained with silver nitrate; and by (iii.) analysis of bone ash for Ca and PO_4 . Spectrographically, the vitamin may be assayed by means of its ultra-violet absorption band.

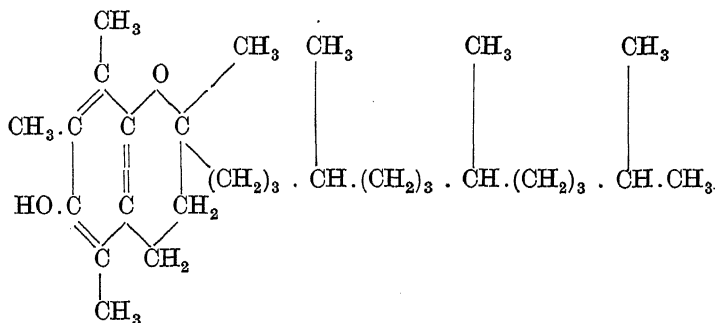
(3) **Vitamins E**, α -tocopherol, $\text{C}_{29}\text{H}_{50}\text{O}_2$, and β -tocopherol, $\text{C}_{28}\text{H}_{48}\text{O}_2$, the placental vitamins, were first recognised as anti-sterility factors by Evans (1922), and by Sure (1923), who observed, independently, that rats reared on purified diets supplemented by vitamins displayed subnormal fertility in the second generation. Fertility could be restored by addition of various natural foodstuffs, notably lettuce, meat, wheat-germ, rolled oats, or milk fat in large amounts.

Sherman has pointed out that the description of vitamin E as the anti-sterility vitamin is misleading, for although it is essential for reproduction it is not any more essential than vitamin A.

Vitamin A deficiency interferes with ovulation, whereas vitamin E deficiency interferes with placental function in the female, and

with germ-cell maturation in the male. The vitamin appears to be a complex alcohol, optically inactive, but with a marked absorption band about $298\text{ m}\mu$. In its natural sources it is accompanied by at least two related compounds with absorption bands at $267\text{ m}\mu$ and $294\text{ m}\mu$. The name α -tocopherol (*tokos*, child-birth, and *phero*, to bear) has been given to the active alcohol, 3 mg. of which administered daily restores fertility to rats on a vitamin E deficient diet.

Structure of α -tocopherol.—Fernholtz has shown that the vitamin is a cyclic ether of hydroquinone, and suggested that it is a derivative of the chromane nucleus. This has been confirmed by synthesis (Smith, Karrer) and biological assay of synthetic chromanes (Evans and Emerson), and it has been shown that the vitamin is a chromane with two substituents, and that other ethers of hydroquinone display vitamin E activity when fed at high levels.



Vitamin E
(α -tocopherol).

In β -tocopherol there are only two methyl groups in the aromatic nucleus.

Sources.—A rich source is the unsaponifiable fraction of wheat-germ oil; other good natural sources are lettuce, watercress, and wheat preparations that include intact germ; moderately good sources are entire cereals, molasses, cotton-seed oil and hemp-seed oil. Animal foodstuffs are poor in the vitamin, and it is absent from liver oils of fish.

Effects of Vitamin E Deficiency.—Neither oestrus cycle nor implantation of ovum is affected, but foetal death occurs at an early stage in development owing to defects in the allantois and yolk sac.

In the human subject, avitaminosis E is a factor in habitual abortion, sterility, both male and female, and excessive hæmorrhage after parturition, though this last may be due to lack of vitamin K. Wheat-germ oil has been administered with success in the

ment of habitual abortion, but the vitamin has not yet been of anticipated value in cases of human sterility. The daily requirement of the vitamin is not yet known, but its limited distribution suggests that its position in the modern dietary is not prominent.

(4) **Vitamin F**, linoleic acid. The designation "F" was originally applied to the B_1 member of the B complex, but has now been used to denote unsaturated acids necessary for growth, reproduction and lactation in rats. Either linoleic or linolenic acid can supply the deficiency, and the former is termed vitamin F by Evans. Vitamin F deficiency was first recorded by Burr, in 1929.

$\text{CH}_3 \cdot (\text{CH}_2)_4 \cdot \text{CH}=\text{CH} \cdot \text{CH}_2 \cdot \text{CH}=\text{CH} \cdot (\text{CH}_2)_7 \cdot \text{COOH}$, Linoleic acid.
 $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}=\text{CH} \cdot \text{CH}_2 \cdot \text{CH}=\text{CH} \cdot \text{CH}_2 \cdot \text{CH}=\text{CH} \cdot (\text{CH}_2)_7 \cdot \text{COOH}$,
 Linolenic acid.

Vitamin F deficiency has not yet been observed in human subjects, and, as Bacharach observes (1937): "The chances of any human diet, however poor, being fat-free are absolutely negligible, and all the available evidence suggests that any fat in the diet is certain to contain some linol(en)ic acid."

(5) **Vitamin K**, the anti-hæmorrhagic vitamin, was recognised, in 1934, by Dam and Schönheyder, who described a deficiency disease in chicks, characterised by anæmia, increased blood-coagulation time, pathological changes in the gizzard, and tendency to hæmorrhage. The condition was traced to the lack of a specific fat-soluble, alkali-labile but thermostable factor, vitamin K, and this conclusion was confirmed independently by Almquist and Stokstad, in 1935.

Sources.—Vitamin K occurs in hog-liver fat, and to a lesser extent in the liver fat of the dog and the cod. Its principle source is the lipid fraction of green vegetables. The vitamin is a yellow oil, soluble in fat solvents and thermostable, but destroyed by alkalis. The unit is defined as the smallest dose, per gm. body weight, which given for three days is sufficient to restore the blood-coagulation time to normal in experimental animals. The low clotting power of vitamin K deficient blood is ascribed to reduction in the prothrombin content of the plasma, and the vitamin is thought to be a prosthetic group, or similar component, of the prothrombin complex. The vitamin does not appear to influence hæmophilia in the human subject.

Two crystalline forms are now known: K_1 , from alfalfa, and K_2 , from fish meal, which is six times as potent.

K_1 is 2-ethyl-3-phytyl-1,4-naphthoquinone, and has been synthesised. Its potency is about 100 units per mg.

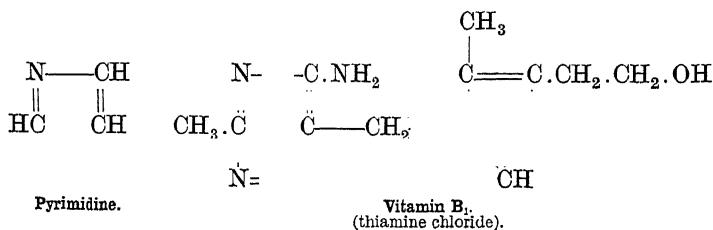
THE WATER-SOLUBLE VITAMINS

The Vitamin B Complex.—Recognition of a group of water-soluble vitamins dates from 1911, when Osborne and Mendel reported the presence of one "growth determinant" in milk free from protein and fat, and another in separated milk fat. McCollum and Davis also reported the presence of two distinct growth factors in natural foodstuffs; one of which they called "water-soluble B" to distinguish it from the other factor, "fat-soluble A." Later on they showed that water-soluble B was the curative of beri-beri, and they concluded it was identical with the "vitamine" that Funk had separated from rice husks, and shown to have anti-neuritic properties.

Resolution of Vitamin B.—The complex can be resolved into a thermo-labile, anti-neuritic factor, B_1 , thermo-stable growth factors, B_2 , B_3 , B_4 , B_5 , and an anti-dermatitic factor, B_6 .

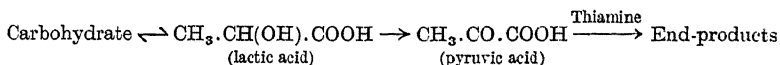
Distribution.—Wheat-germ is rich in B_1 , but poor in B_2 – B_5 ; the converse being true of milk, meat, green leaves, roots, and tubers. Yeasts of equal B_2 content vary greatly in regard to B_1 . Fresh egg-white contains B_2 unaccompanied by B_1 .

(1) **Vitamin B_1 .**—*Aneurin*, or thiamine, $C_{12}H_{17}ON_4SCl$, formerly termed "vitamin F," the anti-neuritic member of the B-complex, has been isolated in crystalline form, m.p. 250° , from yeast and from rice polishings by several groups of investigators (Jansen and Donath, Otake, Windaus, Peters), and has been synthesised by Williams and Cline (1936). The vitamin is a thiazol derivative of 2-methyl-6 amino-pyrimidine, and occurs naturally in the form of a chloride. It is extracted by water or alcohol, an acidified 50 per cent. mixture being the most effective, and may be precipitated by alkaloidal reagents, such as picric acid, and purified by adsorption by charcoal or Fuller's earth. It is stable in neutral or acid solution, but is rapidly inactivated by alkalies at the temperature of boiling water. On oxidation it yields a yellow pigment with a blue fluorescence, *thiochrome*, formerly isolated from yeast, and synthesised by Todd, Bergel and their colleagues (1936).



Sources.—The most potent sources of vitamin B₁ are concentrates prepared from yeast, wheat-germ or rice-polishings. Satisfactory food sources are entire cereals (the vitamin is located chiefly in the germ and the bran), nuts, leguminous seeds and malt extract. Fresh vegetables, fruits and animal products, such as cheese, eggs, fish roe, kidney, liver and milk, are moderately rich in the vitamin.

Functions.—Peters, Kinnersley and their colleagues have shown that thiamine pyrophosphate acts as a co-enzyme in carbohydrate metabolism in nervous and other tissues, and is necessary for the degradation of pyruvic acid, the oxidation derivative of lactic acid. In the absence of the vitamin, pyruvic acid accumulates, and induces the characteristic signs of avitaminosis B₁.



Effects of Vitamin B₁ Deficiency.—(i.) *Beri-beri*.—This disease is endemic among the rice-eating communities of the East, including India, the Malay Peninsula and Japan, where the diet is almost entirely restricted to rice that has been “polished” by removal of the outer husk to improve storage qualities. Beri-beri also appears among wheat-eating communities, such as those of Labrador and Newfoundland, who subsist mainly on refined wheat flour. Infantile beri-beri is a direct consequence of a vitamin deficiency in the diet of the nursing mother, and is responsible for a large part of the enormous infantile mortality of India and the East in general. Beri-beri is a polyneuritis characterised by loss of muscular co-ordination, gradual paralysis of the limbs, alimentary disturbances (indigestion, constipation and colitis), degeneration of the heart muscle, and general emaciation, often associated with dropsy.

Engel and Phillips (1938) claim that the nerve degeneration found in human B₁ deficiency is due to, or aggravated by, lack of other factors, notably flavin and vitamin A.

Eggleton (1939) believes that zinc deficiency is a factor in evoking beri-beri.

(ii.) *Avian Polyneuritis*.—When birds are fed on an exclusive diet of polished rice and water no ill effects are seen for several weeks. Then signs of acute polyneuritis appear rapidly. The bird is unable to fly, to walk, and, eventually, to stand. Exhaustion and death follow in a few days unless the vitamin be supplied. Recovery is dramatic.

The key observation on the association of polished rice with beri-beri and avian polyn neuritis was made by the Dutch physician, Eijkman, when in Java, in 1897. He noticed that domestic fowl fed on the polished rice developed the same type of neuritic disorder as that

found among prison inmates on the same dietary, whereas birds fed on unpolished rice were free from the disease. Similar and independent conclusions were reached by Frazer and Stanton, working in the Malay Peninsula (1909), and by Chamberlain, in the Philippine Islands (1910).

(iii.) *Bradycardia*.—This is a slowing of the heart-beat owing to sinus changes, and responds rapidly to vitamin therapy. It is a feature of infantile beri-beri. The phenomenon has been used by Birch and Harris (1934) for the electrocardiographic assay of vitamin preparations. Rats that have been depleted of vitamin B₁ are fed, under controlled conditions, with graded doses of the test materials, and the resulting cardiac response is observed.

(iv.) *Gastro-intestinal Dysfunction*.—Impairment of appetite, decreased motility of the stomach and loss of tonus, subnormal temperature, anhydræmia, and decreased resistance to fatigue go to make up a syndrome arising from disturbed innervation of the alimentary tract.

(v.) *Pyruvate Accumulation*.—The pyruvate content of blood, urine, and cerebro-spinal fluid rises characteristically in beri-beri, and is specifically reduced by injection of B₁.

Vitamin B₁ Standard and Requirements.—The 1938 International Unit is 3γ of pure thiamine chloride. Sherman's unit, which is about two-thirds of the international unit, is the amount of vitamin required daily to enable a growing rat to gain in body weight by 3 gm. a week over an experimental period of four to eight weeks.

*Vitamin B₁ Value of Common Foodstuffs
(Expressed in International Units per gram)*

Material.	Vitamin B ₁ .	Material.	Vitamin B ₁ .
Beef, raw . . .	0.5	Fruit, fresh . . .	0.3-1.0
Kidney, sheep . . .	0.9	Fig, dried . . .	1.0
" pig . . .	3.4	Raisins . . .	0.75
Liver, ox . . .	1.5	Tomato, pulp . . .	0.4
Fish, raw . . .	0.3	Nuts . . .	0.8-2.0
Milk . . .	0.2	Wheat, entire . . .	2.3-3.4
Cheese . . .	0.0-0.2	" germ . . .	5.9-18.7
Egg-yolk, boiled . . .	1.4	" flour, white . . .	0.0
" white, " . . .	a trace	Oatmeal, dry . . .	3.25
Beans . . .	1.2-1.6	Rice, polished . . .	0.0
Carrot, raw . . .	0.6	" bran . . .	5.6-7.6
Lentil, uncooked . . .	2.1	Coffee bean, ground . . .	1.4
Lettuce . . .	0.9	Cocoa . . .	0
Potato, boiled . . .	0.3	Brewer's yeast, dried . . .	6-23.0

The majority of the above values are from analyses by Baker and Wright (1935). Other rich sources of the vitamin are the

commercial preparations *Aluzyme*, *Marmite* and *Bemaz*, and various malt extracts.

The adult human requirements have been assessed by Jung (1932) at 200–300 international units, *per diem*.

Vitamin B₁ in Plant Growth.—Thiamine is essential in plant metabolism. Some green plants and some fungi are autotrophic, in that they synthesise sufficient for their complete requirement. Others are heterotrophic in regard to thiamine, and are represented by many parasitic types, bacteria, yeasts and fungi, the growth of which requires an external supply of the vitamin. R. Williams regards the universal necessity for thiamine as proof of a common chemical ancestry of all forms of life.

Functions of Vitamin B₁.—Thiamine pyrophosphate associated with one protein as co-carboxylase co-acts in the decarboxylation of pyruvic acid to acetic aldehyde and carbon dioxide, and is part of the mechanism for liberating carbon dioxide in plant metabolism. Conversely, it may be concerned in the photo-synthetic fixation of carbon dioxide by an aldehyde acceptor. Associated with another protein, it co-acts in the simultaneous dehydrogenation and decarboxylation of pyruvic acid to acetic acid. In the animal, thiamine is necessary for the complete metabolism of glucose beyond the stage of pyruvic acid.

Tests for Vitamin B₁.—Biological tests include (i.) the cure of retracted neck in pigeons after a twenty to thirty days diet of polished rice and water; (ii.) the cure of convulsive paralysis and (iii.) bradycardia in rats on a deficiency diet.

Chemical tests include (i.) oxidation by alkaline ferricyanide to thiochrome (p. 354), the concentration of which can be measured from its fluorescence in ultra-violet light; (ii.) the formaldehyde azo test (Kinnersley and Peters, 1938).

(2) **Vitamin B₂** (vitamin G).—The anti-dermatitic and water-soluble growth factor has been resolved into two components: riboflavin and an associate, vitamin B₆ or the “Y-factor.” Riboflavin is a member of the lyochromes, or water-soluble yellow pigments that impart a greenish-blue fluorescence to various plant and animal extracts. Riboflavin has been obtained in crystalline form from egg albumin, yeast, liver, kidney and urine. Its structure and chemical relationships are given on p. 206. Both natural and synthetic riboflavin have the growth-promoting properties characteristic of vitamin B₂ preparations, but are devoid of anti-dermatitic and pellagra-preventing properties.

Sources.—The chief sources of vitamin B₂ complex are concentrates from yeast and from whey. Important food sources are:

yeast, animal organs, including liver, kidney and spleen, lean meat, egg-white, milk, and some vegetables, such as beet and turnip greens, spinach, potato and watercress. The partition of the two components, however, differs in the various foods. Thus, as regards

Riboflavin (Vitamin B₂) Content of Foodstuffs
(Fixsen and Roscoe, 1938)

Material.	Riboflavin in mg. per 100 gm.
Cereals :	
Barley, unsprouted . . .	0.01
sprouted, 1-8 days . . .	0.1-0.22
Maize	0.1
Malt extract	0.1
Wheat, whole	0.02
germ	0.033
Fruits :	
Banana	0.0075
Orange, juice	0.007 per 100 ml.
Vegetables :	
Cabbage, white	0.05
Carrot	0.02
Grass, fresh	0.05-0.142
dried	0.6
Potato	0.01
Spinach, fresh	0.057
Animal products :	
Milk, cow's	0.1 per 100 ml.
Egg, hen's, yolk	0.55
white	0.45
Meat, ox brain	0.1-0.5
ox kidney	0.8-2.0
ox liver	0.1-2.4
ox suprarenal	0.5-1.0
rabbit heart	0.55
rabbit kidney	1.3
rabbit liver	0.64
rabbit lung	0.89
Miscellaneous :	
Beer, dark	0.029 per 100 ml.
Honey, pine	0.106
Yeast, dried, brewer's	1.8-3.0
baker's	2.5-3.6
" Marmite "	3.30

both factors, ox liver is a potent source ; beef heart and muscle, veal and chicken are good sources ; and milk is a fair source. Fish muscle and liver is rich in B_6 , but almost devoid of riboflavin. Egg-white contains riboflavin, but has very little B_6 .

Functions.—Riboflavin phosphate, when combined with a protein, forms the respiratory carrier, or "yellow catalyst" of Warburg, which takes part in the dehydrogenation of glucose, lactic acid and other important intermediate metabolites. Hence, riboflavin is probably required for the growth and maintenance of every animal tissue.

The daily requirement of vitamin B_2 appears to be proportional to the food intake, and Drummond suggests that this is because the vitamin is concerned both in amino acid deamination and in urea formation, which would explain the fact that both liver and kidney are very rich in riboflavin. The human requirement for normal health is estimated to be 40 to 50 γ of riboflavin per 100 kilocalories of food (Sherman and Lanford, 1938; Bessey, 1938).

(3) **Vitamin B_6 , adermine.**—A dermatitis occurs in rats fed on experimental diets, and somewhat resembles human pellagra. It is cured specifically by a factor which accompanies the B complex in yeast, cereal germ and bran, muscle, liver and milk. B_6 is a pyridine derivative, and has been synthesised by Keresztesy and Stevens (1939). While necessary for the growth and health of rats, its significance in the dietary of the human subject awaits demonstration.

Residual Vitamins of the B Complex.—At least a dozen different accessory food factors have been referred to the B group in natural food sources, especially yeast. These include B_3 , a thermo-labile avian growth factor (Williams and Waterman); B_4 , an alkali-labile rat growth factor (Readers); and B_5 , a thermo-stable and alkali-stable rat growth factor (Carter); H, an anti-dermatitic factor (Kuhn), identified with factor Y (Chick and Copping) and vitamin B_6 . Rats require at least six different factors from the B complex : including thiamine, riboflavin and adermine.

The gradual resolution of the vitamin B complex has introduced a new concept into nutrition ; namely, the existence of species differences in the growth requirements of higher animals. Thus, B_1 is necessary for birds and mammals ; B_2 and B_4 are necessary for mammals, while B_3 is necessary only for birds.

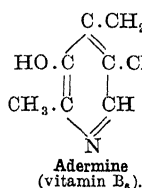
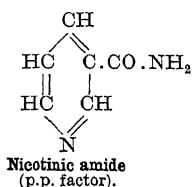
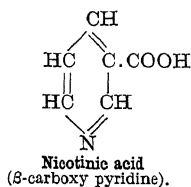
This species difference may be associated with the fact that birds and mammals differ profoundly in their nitrogenous metabolism ; the bird being *uricotelic*, and excreting waste protein nitrogen as uric acid, whereas the mammal is *ureotelic*, and excretes nitrogen chiefly as urea (p. 378).

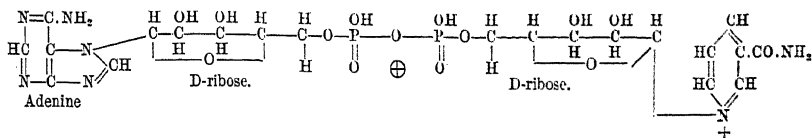
Panthothenic Acid, a derivative of β -alanine, is widely distributed in living tissue, and has been identified with an avian anti-dermatitic factor found in yeast (Woolley, 1939).

Vitamin B Refection.—Spontaneous recovery from avitaminosis $B_1 + B_2$ while on a deficiency diet has been observed, especially among rats, and is accompanied by a marked change in the bacterial flora of the intestine. It is due to local synthesis of the vitamins by micro-organisms in the lower alimentary tract, and is termed *refection*. It is favoured by a high starch content in the deficiency diet. Rfection is very important, and may introduce a serious error into methods of biological assay.

(4) **The Pellagra-preventing Factor.**—Vitamin B_2 was provisionally defined as “the more heat-stable, water-soluble dietary factor necessary for growth and health and prevention of characteristic skin lesions,” among which was often included pellagra.

Pellagra, or “rough skin,” is a disease common in Rumania, South Russia, the southern States of America, and in Italy. Described by Aykroyd as “perhaps the most horrible of all food deficiency diseases,” it is characterised by dermatitis, pigmentation and thickening of the skin, inflammation of the tongue and intestinal tract, and nervous disorders leading to atrophic paralysis and dementia. Some of the signs resemble the condition of “black tongue” found in dogs on a diet lacking vitamin B complex. The pioneer work of Goldberger (1910–30) showed that endemic pellagra was a poverty disease and could be abolished by the use of fresh meat and green vegetables, which he found to contain a pellagra-preventing, or p.p. factor, now identified as nicotinic acid (Spies, *et al.*; Elvehjem, *et al.*, 1937). Nicotinic acid was isolated by Funk in 1911 from rice bran, but its significance was overlooked, as it did not possess the growth-promoting properties of a vitamin. Nicotinic acid is β -carboxy pyridine, and is a constituent of the alkaloids nicotine and trigonelline. Its amide is a constituent of the co-enzymes co-dehydrogenase I and II, which may account for the importance of nicotinic acid in animal nutrition. The effective daily dose in the cure of human pellagra is 0.5 gm. Nicotinic acid may be regarded as the pro-vitamin.





Co-dehydrogenase I (co-enzyme I, co-enzyme)
(adenosine-ribose-diphosphoric-ribose-nicotinic amide).

Co-dehydrogenase II has a similar formula but for the insertion of an additional —PO(OH)O— in the chain between —O— and —P— at the point \oplus , making a triphosphoric linkage.

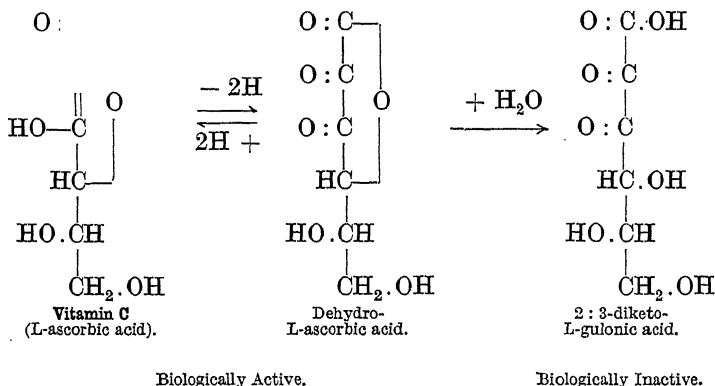
(5) **Vitamin C, Ascorbic Acid.**—The anti-scorbutic properties of fresh vegetables and citrus fruits (lemon, lime and orange) were known to the Dutch sailors in the fifteenth century, and during the reorganisation of the British Navy by Jervis and Nelson, lime juice was included in the service dietary. The remedy proved of some value at a time when disease was more dangerous than gun-fire, but even as late as 1916 it was believed by many that scurvy arose from infection. Between 1907 and 1912, Holst and Frölich produced experimental scurvy in guinea pigs by keeping them for two to three weeks on a diet of oats and bran, and showed that the condition resembled human scurvy in all respects, and, like it, was cured promptly by fresh fruits or vegetables. The anti-scorbutic material was fractionated by Bezssonoff, King, Zilva, and other workers, who found that it was associated with the power of decolourising the indicator dye, 2:6-dichlorophenol indophenol, and Tillmans, in 1932, suggested that the vitamin was the actual reducing substance. Vitamin C was obtained from lemon juice by Waugh and King in 1931 and proved to be identical with *hexuronic acid*, $\text{C}_6\text{H}_8\text{O}_6$, isolated, in 1928, from adrenal cortex by Szent-Györgyi, who later recognised its anti-scorbutic properties. The structure was elucidated by Haworth, and confirmed by two independent syntheses, in 1933 (Hirst and Haworth, Reichstein), and the vitamin was renamed *ascorbic acid*.

Properties.—Ascorbic acid, $\text{C}_6\text{H}_8\text{O}_6$, m.p. 192° , $[\alpha]_D^{25} = +24^\circ$, is a colourless compound, very soluble in water, and possessing strong reducing properties. The cold solution is able to bleach indicator dyes, reduce Fehling's reagent, and blacken silver nitrate. This last reaction has led to the suggestion that ascorbic acid is responsible, in part at least, for the local silver staining observed in the "argentophil" inclusions present in many cells. Ascorbic acid is the most unstable of all the vitamins. It readily undergoes reversible oxidation followed by hydrolytic destruction, especially in warm, alkaline solutions. This instability explains why lemon juice and lime juice, after boiling, bottling and storing until they had

acquired "vintage" properties, may be useless as anti-scorbutic remedies.

Fresh fruits and vegetables have been shown by Zilva to contain an enzyme, *ascorbic oxidase*, which contributes to the disappearance of the vitamin when plant products are stored.

On oxidation, ascorbic acid loses two hydrogen atoms and is converted into *dehydro-ascorbic acid*, which is still biologically active, and can be reconverted into the vitamin by reducing agents, such as H_2S . In neutral or alkaline solution, dehydro-ascorbic acid is spontaneously hydrolysed to 2:3-diketo-L-gulonic acid, a derivative of the sugar L-gulose. This acid has no anti-scorbutic properties, and cannot be reconverted into the vitamin by reduction. Since the vitamin is derived from the L-sugar, it is termed L-ascorbic acid. Its isomer, D-ascorbic acid has been synthesised and shown to be biologically inactive.



Both forms of ascorbic acid are lactone derivatives of the substituted gulonic acid. When the lactone ring had been opened by hydrolysis, the vitamin is irreversibly inactivated.

Sources.—The synthetic vitamin is now obtainable commercially. Other highly potent sources are concentrates prepared from lemon or orange juice. Natural food sources are represented by citrus fruits and raw vegetables, especially tomato, celery, onion and rhubarb. The best animal sources are liver and fresh milk.

Milk as secreted contains the vitamin in the reduced form, but it is soon inactivated. Kon and Watson (1936) report that a pint bottle of milk exposed to sunlight for half-an-hour, and then kept in darkness for an hour, had lost at least half of its original anti-scorbutic properties.

Hopkins (1938) has shown that lactoflavin in presence of light catalyses the oxidation of ascorbic acid, and thus is responsible for its spontaneous destruction in milk.

The vitamin is present in almost every part of higher plants and animals, being maximal in glandular tissue, and minimal in muscle and storage fat. It is formed rapidly in germinating seeds, which provide a valuable emergency source of the vitamin; a fact unknown to the defenders of Kut in 1916. Its precursor in plants appears to be mannose.

Functions.—The reversible oxidisability of the vitamin suggests that it can act as a hydrogen-transport agent in cell metabolism, and since its formation precedes that of chlorophyll and the carotinoids in the growing plant, it is probably concerned in the mechanism for synthesising these pigments.

Effects of Vitamin C Deficiency.—(i.) *Infantile Scurvy.*—This condition is found in children fed exclusively on sterilised or artificial dietaries, or by mothers whose milk is deficient in ascorbic acid. After a period of anæmia and irregular growth, the characteristic syndrome appears: sore gums, periosteal hæmorrhages at the joints, subcutaneous hæmorrhages and hæmaturia.

(ii.) *Adult Scurvy.*—This is seen among explorers and others on dietaries deprived of fresh foodstuffs for prolonged periods, and is always a potential danger in communities living on artificially modified foods, especially when individual requirements are increased by pregnancy, lactation or disease. The scorbutic syndrome includes hæmorrhages from mucous membranes, skin, joints, limbs and bone marrow, spongy and bleeding gums, pain and swelling in joints and limbs. The disease progresses to complete incapacitation, and terminates fatally.

Many of the signs of scurvy are referable to increased permeability and escape of blood into the tissues.

(iii.) *Dental Disorders.*—Spongy and bleeding gums, decay and loosening of the teeth, pyorrhœa: all have been attributed to avitaminosis C, and have been cured or checked by ascorbic acid. The vitamin appears to be necessary for maintaining the activity of the formative cells, odontoblasts, ameloblasts and osteoblasts.

(iv.) *Rheumatism.*—The lack of a dietetic factor in rheumatic conditions has often been suggested, and some of the signs and symptoms of prolonged subacute avitaminosis C closely resemble rheumatoid states. Therapeutically, ascorbic acid has not proved to be a specific cure.

Vitamin C Standard and Requirements.—The 1934 International Standard is the biological activity of 0.05 mg. of pure L-ascorbic acid, which now replaces the former standard, 0.1 ml. of fresh lemon juice. Sherman's unit is the daily amount of vitamin just sufficient to protect a standard guinea pig from the effects of a scorbutic diet. Sherman's unit is approximately equal to 10 international units.

The minimal daily requirement to prevent any possible prescorbutic condition in man is about 19–25 mg. ascorbic acid, the advisable intake for a 70 kg. subject being about 60 mg., or nearly 1 mg. per kg. body weight. The requirements of a child are about twice as much. Many animals, notably the rat, are immune from scurvy, and presumably can synthesise the vitamin. Man, the monkey, and the guinea pig, are entirely dependent on exogenous sources.

On an ordinary mixed diet, 25–30 mg. of ascorbic acid are excreted daily in the urine. The animal organism is unable to store the vitamin beyond a tissue saturation limit, which is, generally, less than 0.5 mg. per gram. Blood plasma ascorbic acid ranges from 0.8 to 2.4 mg. per 100 ml., the average being 1.6. In scurvy the plasma has a value of 0.4, or less.

(6) **Vitamin P.**—In 1936, Szent-Györgyi and his colleagues found that lemon juice was effective in curing capillary hæmorrhage of the purpura type, whereas ascorbic acid was not. The agent is a flavone glucoside and functions by increasing the resistance of capillary walls to the application of pressure. It may be obtained from lemon or orange peel, and is present in the commercial preparation “citrin.”

Vitamin C Value of Common Foodstuffs
(Expressed in mg. ascorbic acid per gm. or per ml.)

Material.	Ascorbic Acid.	Material	Ascorbic Acid.
Orange juice .	0.22–0.89	Milk, cow .	0.02–0.03
Lemon juice .	0.47–0.73	human .	0.10–0.15
Tangerine juice .	0.10–0.78	Egg-yolk .	0.00
Marmalade .	0.06–0.14	Liver, ox .	0.68
Tomato juice .	0.17–0.7	Spinach, raw .	1.2–4.8
Apple juice .	0.02–0.1	boiled .	0.2–0.3
Grape-fruit juice .	0.59–0.65	Cabbage, raw .	0.8–1.4
Strawberry .	0.17–0.6	boiled .	0.01–0.09

Bacharach and his colleagues, from whose work many of the above data have been taken, report that storage for a month causes a 20 per cent. loss of ascorbic acid in oranges, and a 6 per cent. loss in lemons. The loss, however, was negligible in juices properly concentrated and stored. The vitamin C value of cooked vegetables depends largely on the time and condition of the cooking. Where sodium bicarbonate is added to improve the colour, and the boiling is prolonged, the ascorbic value drops to zero. Rapid and brief sterilisation of milk by heating to about 90° (pasteurisation) has little effect on the vitamin, boiling for more than ten minutes may complete the inactivation that has been in progress ever since the milk was secreted.

Estimation of Ascorbic Acid.—This may be done spectrographically, from the extinction coefficient of the characteristic

absorption band at 245 m μ , or by titration with a suitable oxidation indicator.

Many animal tissues contain reducing substances, notably adrenaline, glutathione, and cysteine, and the specificity of the reduction method for estimating vitamin C was uncertain until it was shown that the redox indicator 2 : 6 dibromophenol-indophenol is not affected in acid solution by adrenaline or glutathione, although it is rapidly reduced by ascorbic acid. Furthermore, mercuric acetate does not precipitate ascorbic acid, although it removes other reducing factors, including glutathione and cysteine. By adaptations of these methods, Harris and other workers have been able to estimate the ascorbic acid content of various substances and to show that it parallels the vitamin C content, as determined biologically.

Oxidised ascorbic acid does not reduce the indicator, so the mixture is treated first with H₂S, which both reduces the dehydroascorbic acid, and removes any excess of mercury that may remain from the preliminary purification.

On addition of a few drops of 0.2 per cent. cacothelin to ascorbic acid in 0.2 per cent. HCl, a lilac colour develops in a few minutes. Cysteine and glutathione only react on boiling. The test will detect 0.01 per cent. ascorbic acid, or less, if the concentration of the HCl be increased (Rosenthaler 1938).

Capillary Test (Göthlin, 1931-38).—Compress the upper arm for 15 minutes at a pressure of 50 mm. Hg. by means of a sphygmomanometer cuff. If the number of petechial spots that appear in a circle 60 mm. in diameter, drawn on the front of the elbow, exceeds six, the subject has increased capillary fragility, which is characteristic of avitaminosis C. Bell (1939) tests both arms simultaneously at a distance of 2 feet from a 300-watt lamp. He regards eight spots per circle as being the upper normal limit, but notes that a small group of subjects (5 in 346) displayed a high fragility, unaffected by administration of vitamin C or P.

MISCELLANEOUS FACTORS IN THE DIET

The Hæmopoietic Factor.—From measurements of the red cell constants, diameter, volume, iron and hæmoglobin content, it is possible to classify anæmias into two groups : (1) the hyperchromic macrocytic type, in which the cells are large and fully saturated with hæmoglobin, and (2) the hypochromic microcytic type, in which the cells are small and deficient in hæmoglobin. Hypochromic anæmia is essentially an iron-deficiency disease, and responds to iron and copper therapy. Hyperchromic anæmia, which

may occur in pernicious form, is due to lack of a specific hæmatogen (the extrinsic factor) in the diet, or absence of an enzyme (the intrinsic factor) which is present in normal gastric juice, and converts the hæmatogen into a *hæmopoietic factor*, which is stored in the liver. The extrinsic factor closely accompanies the vitamins of the B group in distribution, and may be obtained plentifully from rice-polishings, wheat-germ, ox heart and muscle, and autolysed yeast preparations. It is absent from many samples of dried yeast.

A hæmopoietic factor has been isolated from liver by Dakin and West (1935), and is a hexosamine peptide, now available commercially under the name of "anahæmin."

Choline, $C_5H_{15}O_2N$, acquired a significance in nutrition when Best and Huntsman in 1932, showed that it had both a curative and a preventive action on the excessive accumulation of fat in the mammalian liver. Choline is a normal constituent of the mixed human dietary, being derived from lecithin and the other phospholipides present in animal foodstuffs rich in fat. Lecithin contains about 14 per cent. of choline, and its principal sources are egg-yolk, liver, kidney, heart and pancreas. Human milk has a lecithin percentage of 0.02-0.08, while cow's milk has 0.06-0.11. Betaine, the nitrogenous base found in beetroot and other vegetable sources, has a similar effect on hepatic liposis as choline, and it has been suggested that both these compounds be included among the water-soluble vitamins.

Dietary Standards.—Among the most important of the modern dietaries are those drawn up by Stiebling and Ward for the United States Department of Agriculture, in 1933, and the British nutritional standards prepared by the Ministry of Health in 1934, which conform with the Stiebling scale.

Daily Food Requirements (Stiebling)

Subject.	Energy, kilocal.	Protein, gm.	Ca, gm.	P, gm.	Fe, mg.	Vitamin A.	Vitamin C.
						International Units.	
Child under 4 years .	1,200	45	1.0	1.0	6-9	1,500	1,085
Boy, 4-6; girl, 4-7 .	1,500	55	1.0	1.0	8-11	1,500	1,160
Boy, 7-8; girl, 8-10	2,100	65	1.0	1.0	11-15	1,750	1,230
Boy, 9-10; girl, 11-13	2,400	75	1.0	1.2	12-15	1,750	1,300
Woman, moderately active	2,500	75	1.0	1.2	13-15	2,000	1,370
Boy, 11-12; girl, 13	2,500	75	1.0	1.2	13-15	2,000	1,370
Woman, very active .	3,000	75	0.88	1.32	15	2,000	1,450
Active boy, over 15 .	3,500	75	0.88	1.32	15	2,000	1,450
Man, moderately active	3,000	67	0.68	1.32	15	2,000	1,450
Man, very active .	4,500	67	0.68	1.32	15	2,000	1,450

The calorie requirements of the British standards are similar to those prescribed by Stiebling; the protein requirement is specified as 80–100 gm. *per diem*, of which not less than one-third must be of animal origin.

Protective and Non-protective Foods.—McCollum has drawn a practical distinction between protective foods, such as animal fats, liver oils, milk products, eggs, fresh fruits and green vegetables, and non-protective foods, such as bread and other cereal preparations, sugar, root vegetables and vegetable fats, including margarine. Both classes provide calories, but the protective foods are richer in proteins of high biological value, vitamins and biological elements, and should be used to supplement and correct the dietary.

GENERAL REFERENCES

- ABRAHAMS, M., and E. M. WIDDOWSON (1937), "Modern Dietary Treatment." London.
- ADOLPH, E. F. (1933), "Metabolism and distribution of water." *Physiol. Rev.*, **13**, 336.
- AYKROYD, W. R. (1936), "Vitamins and other Dietary Essentials." 2nd Ed. London.
- AYKROYD, W. R. (1937), "Human Nutrition and Diet." London.
- BACHARACH, D. L. (1938), "Science and Nutrition." London.
- CLARE, F., and R. M. TITMUS (1939), "Our Food Problem," London.
- DRUMMOND, J. C., and A. WILBRAHAM (1939), "The Englishman's Food." London.
- FEARON, W. R. (1936), "Nutritional Factors in Disease." London.
- FIXSEN, M. A. B., and M. H. ROSCOE (1938), "Tables of the vitamin content of human and animal foods." *J. Nutrition*, **7**, 823.
- HARRIS, L. J. (1938), "Vitamins and Vitamin Deficiencies." Vol. I. London.
- HESS, A. F. (1929), "Rickets." Philadelphia.
- KING, C. G. (1926), "Vitamin C, ascorbic acid." *Physiol. Rev.*, **16**, 238.
- LEITCH, I., and J. DUCKWORTH (1938), "Protein requirements of man." *J. Nutrition*, **7**, 257.
- LUSK, G. (1928), "Science of Nutrition." 4th Ed., London.
- MELLANBY, E., (1934), "Nutrition and Disease." London.
- MELLANBY, M. (1937), "Nutrition as a factor in resistance to dental caries." *British Dent. J.*, **62**, 241.
- ORR, J. B., and I. LEITCH (1938), "Calorie requirements of man." *J. Nutrition*, **7**, 509.
- SHERMAN, H. C. (1937), "Chemistry of Food and Nutrition." 5th Ed., London.
- STERN, F. (1937), "Applied Dietetics." London.
- WILLIAMS, R. R. (1938), "Chemistry of vitamin B₁." *Ergeb. Vitamin- und Hormonforschung*, **1**, 213.

CHAPTER XV

ALIMENTARY DIGESTION

digestion is the process whereby the ingested food materials are rendered *assimilable*, or capable of passing through the intestinal mucosa into the portal blood stream or the lymphatic system. In general, it consists of a series of zymolytic hydrolyses that convert the colloidal food molecules into simple, non-colloidal solutes.

Digestion cannot be studied adequately apart from physiology. It is a complex process, involving nervous and chemical co-ordination of secretion and muscular movement.

Three stages may be recognised: *oral*, *gastric*, and *intestinal*. Each is associated with the presence of specialised secretions.

ORAL DIGESTION

The digestive secretion is saliva, the mixed product of three pairs of glands. It is a lubricant for the mouth cavity, a solvent, an adhesive for food material, and also has a hydrolytic action on starch, which may persist for some time after the food has reached the stomach.

Mixed saliva has a reaction of pH 5.7–pH 7.0; the usual range being pH 6.35–pH 6.85. This slight acidity may be increased by fermentation of carbohydrates in an infected mouth, and for this reason morning saliva is often the more acid. The total solids amount to about 0.6 per cent., but wide variations occur. Parotid saliva is usually more dilute than sublingual or submaxillary saliva. The total volume secreted by the normal adult in twenty-four hours is believed to be as high as 1,500 ml.

Mixed Human Saliva (Representative Composition)

Organic Solutes, per cent.		Inorganic Solutes, Mg. per 100 ml.	
Mucin .	0.2–0.3	K ⁺ .	30–100
Ptyalin .	+	Na ⁺ .	20–30
Urea .	0.02	Ca ⁺⁺ .	8
Thiocyanate	0.00–0.1	Mg ⁺⁺ .	1
		Cl ⁻ .	40–50
		H ₂ PO ₄ ⁻ .	10–60

Identification of the Constituents of Saliva.—(i.) *Ptyalin* (salivary amylase). Add 1 ml. of saliva to 10 ml. of neutral 1 per cent. starch solution. Mix and incubate at 40°–50° C. in a water-bath. Every ten minutes withdraw a few drops of the mixture and add to a test tube containing 5 ml. of water and 1 drop of iodine (cf. p. 118).

As zymolysis proceeds, the opalescence of the starch solution clears, and the colour reaction with the iodine changes from blue to red-brown (dextrin stage), and, finally, addition of the iodine causes no colour change (sugar stage). The original mixture will now be found capable of reducing Fehling's or Benedict's reagent, owing to production of maltose from the starch. *Ptyalin* is the characteristic enzyme of saliva; it acts on starch and glycogen, converting them ultimately into maltose. Carbohydrate food is not kept long enough in the mouth for this change to take place, but the zymolysis continues in the stomach until the saliva is inactivated by the acid of the gastric juice. *Ptyalin* is not essential for digestion, and is absent from the saliva of many animals.

(ii.) *Mucin*.—Apply the protein copper test and the arginine test to saliva. Positive results are obtained indicating the presence of protein. To identify the protein, acidify 3–5 ml. of saliva with a few drops of 20 per cent. acetic acid as long as a precipitate forms. The precipitate is glycoprotein, and does not dissolve in excess of the acid, which distinguishes it from nucleoprotein.

Mucin is a mixture of glycoprotein and mucopolysaccharide. It acts as a lubricant and protective agent for the alimentary tract.

(iii.) *Thiocyanate*.—Add a drop or two of dilute (1 per cent.) ferric chloride to about 2 ml. of saliva. An orange-red colour developing shows the presence of thiocyanate. Or add a few drops of saliva to a filter paper that previously has been dipped in ferric chloride solution and dried. This is a more convenient way of carrying out the test.

Thiocyanate or sulphocyanide is secreted as the potassium salt, KSCN. The amount is variable and often it is absent.

Speculation has ascribed several functions to salivary thiocyanate: (a) an oral antiseptic, (b) an activator of *ptyalin*, (c) a form in which —CN compounds are eliminated, (d) a by-product of sulphur metabolism, possibly related to the taurine of bile.

(iv) *Nitrite*.—Add a couple of drops of a 2 per cent. alcoholic guaiacum solution to about 2 ml. of saliva. Acidify with 2 drops of glacial acetic acid, and shake. A blue colour develops if the saliva contain nitrite.

This test must not be confused with the familiar guaiacum-peroxide test for blood (p. 403), which will not work in an acid solution. Many

other reagents, such as α -naphthylamine, may be substituted for the guaiacum with better results.

Saliva usually contains not more than 1–2 parts HNO_3 per million.

Ammonia and *nitrite* occur in traces in most samples of saliva from the mouth but not in catheter specimens fresh from the parotid duct. They arise from the bacterial decomposition of food residues, and their concentration is an index of oral infection.

(v) The *chloride* content of saliva is usually a little less than that of the blood plasma. It is greatly increased in conditions of chloride retention, especially those associated with acute nephritis.

(vi) *Potassium*.—Unlike the usual tissue fluids, saliva is richer in potassium than in sodium; the excess being excreted as thiocyanate.

(vii) *Calcium* occurs chiefly in submaxillary and sublingual saliva, and aids in the formation of scale deposits on the teeth of the lower jaw. Calcium may function as an activator of ptyalin.

(viii) *Urea* is present in saliva by simple diffusion from the blood stream, as it is a very mobile solute. The urea content of saliva is about 10 per cent. lower than that of the blood, and may be estimated as a substitute for blood urea estimations, but the method is not entirely satisfactory, as there are wide variations in the composition of mixed saliva.

GASTRIC DIGESTION

Gastric juice has a double origin, the organic solutes being derived from the glandular epithelium of the fundal and pyloric regions of the stomach, while the free hydrochloric acid is secreted by the parietal or border cells found in the glandular epithelium of the fundal region.

Parietal secretion is evoked by histamine injection, and is essentially an isotonic solution of HCl , approximating in value to 0.1 N, or 0.365 per cent. Non-parietal secretion is evoked by pilocarpine injection, and is rich in nitrogenous solutes and neutral chloride, but poor in free HCl . As collected, gastric juice may have a concentration of HCl up to 0.1 N, in man; and 0.17 N, in dogs. The pH range is 1.1–1.8.

Significance of the Constituents of Gastric Juice.—(i.) *Pepsin* is a proteoclastic enzyme that in acid solution converts higher proteins first into acid metaproteins, and then into peptides. It is unable to attack keratin, and for this reason drugs intended to act in the intestine are sometimes administered in keratin capsules so as to pass through the stomach unchanged.

(ii) *Rennin* converts soluble caseinogen into insoluble casein

Percentage Composition of Mixed Gastric Juice

Organic Solutes, 0·04–0·15.		Inorganic Solutes, 0·08–0·5.	
Pepsin	+	Total Cl	. . 0·3 –0·5
Rennin	+	Free HCl	. . 0·2 –0·36
Lipase	+	Na ⁺	. . 0·05–0·07
Hæmatinic factor	+	K ⁺	. . +
Mucin	+	Ca ⁺⁺	. . +
		Mg ⁺⁺	. . +
		NH ₄ ⁺	. . ±

(paracasein). It is an important enzyme in the stomach of young mammals, as by coagulating the ingested milk it retains casein in the stomach and thus prolongs the action of pepsin.

(iii) *Lipase*, the esterase that splits fats, does not find its optimal pH in the stomach, and does not act much on fat during gastric digestion.

(iv) *Free Hydrochloric Acid*.—This constituent distinguishes gastric juice from all the other animal secretions. The concentration is usually such that one volume of pure juice will neutralise the same volume of N/10 NaOH, the result being expressed as the *titration value*. During digestion the acidity is decreased by union with digestion products, and the value of ordinary gastric contents is such that 100 ml. are neutralised by 5–50 ml. of N/10 NaOH.

Gastric HCl has several functions : it is necessary for the action of pepsin ; it hydrolyses (inverts) sucrose, maltose, and lactose ; it inactivates salivary ptyalin ; it aids in the control of the pyloric sphincter ; and it is an important gastric antiseptic.

When the concentration of HCl is low, its antiseptic action is weak, and secondary fermentations are liable to occur in the stomach. These are due to organisms swallowed along with the food, and the usual end-products are butyric acid and lactic acid (derived from carbohydrates). Butyric acid causes the characteristic sour smell of regurgitated gastric contents ; lactic acid has no odour.

Identification of the Constituents of Gastric Juice

(1) **Pepsin**.—Add sufficient washed fibrin or coagulated egg-white to fill the rounded end of each of three test tubes, *a*, *b* and *c*. To *a* add 5 ml. of gastric juice and 1 ml. 0·1 N HCl. To *b* add 5 ml. of gastric juice and enough 0·1 N NaOH to make it alkaline to phenol red. To *c*, the control tube, add 5 ml. of water and 1 ml. of 0·1 N HCl. Label the tubes, and incubate at 40–50°. Examine

after thirty to forty-five minutes. The presence of the proteoclastic enzyme pepsin is shown by the gradual dissolution of the protein in tube *a*. The protein in tube *b* is not attacked because pepsin will only work in an acid medium.

(2) **Rennin.**—Add 5 drops of gastric juice to 5 ml. of fresh milk. Incubate at 40–50°. The milk is converted into a solid coagulum if the juice contains rennin. Excess of juice must be avoided as the acid present will precipitate the caseinogen, and thus simulate the formation of a clot.

(3) **Hydrochloric Acid.**—(*a*) *Gunzberg's Test.*—Evaporate to dryness in a porcelain dish or, with precautions, on a filter paper, a mixture of 2–4 drops of gastric juice and 2–4 drops of Gunzberg's reagent (2 gm. of phloroglucinol and 1 gm. of vanillin in 100 ml. of alcohol). Avoid charring the mixture. If the juice contains free HCl, a carmine stain appears when the mixture has dried. The colour disappears on cooling, and reappears on heating.

Similar colours are given by the strong acids HNO_3 and H_2SO_4 , but these are never present in gastric juice. Lactic, butyric, and similar weak organic acids give no colour with the reagent.

(*b*) *Indicator Tests.*—About one to two hours after digestion has begun, gastric contents are strongly acid liquids, with a pH value below 2, and react accordingly with appropriate indicators. To 3 ml. of gastric juice or contents add 5 drops of 0.1 per cent. *methyl-violet*, and note the colour. Repeat the test, using 0.1 per cent. *thymol blue*, and 0.1 per cent. *tropæolin 00*.

Indicator.	Colour.	pH.	Inference for Gastric Contents.
Methyl violet . .	green.	1.0–1.5	hyperacid.
Methyl violet . .	blue.	1.5–2.5	normal range.
Methyl violet . .	violet.	3	hypoacid.
Thymol blue . .	red.	1.4	hyperacid.
Thymol blue . .	orange.	1.5–2.5	normal range.
Thymol blue . .	yellow.	2.8	hypoacid.
Tropæolin 00 . .	pink.	2	hyperacid or normal.
Tropæolin 00 . .	orange.	2.0–2.5	normal range.
Tropæolin 00 . .	yellow.	3	hypoacid.

For exact work it is necessary to match the colours with standards of known pH in a comparator.

Notes on the Indicators.—*Methyl violet*, also known as “crystal violet” and “gentian violet,” is unsuitable for titrations as it is rapidly bleached in acid solutions. The indicator is convenient for rough work, and may be obtained in the form of the ordinary copying-ink pencil, the “lead” of which is dissolved in water.

Thymol blue is a triple-change indicator ; below pH 1.4 it is *red*, between pH 2.8 and pH 8 it is *yellow*, and above pH 9.4 it is *blue*. Hence it may be used for the double titration of free acid and acid salts in gastric contents. Thymol blue has the disadvantage of being affected in acid solution by mucin and by products of protein digestion (peptones and peptides), and may fail to give an accurate indication of acids when it is applied in the later stages of gastric digestion.

Töpfer's reagent (*methyl yellow*, dimethylamino-azobenzene) is used often as an indicator of gastric acidity. Its colour change resembles that of tropæolin 00, but its transition range is between pH 2.9 (*red*) and pH 4 (*yellow*), which makes it unsuitable for the detection of high values of free HCl.

Tropæolin-phthalein, or "t.p." indicator is prepared by mixing equal volumes of 0.1 per cent. tropæolin 00 and phenol phthalein. Like thymol blue, it has a triple change ; *red* below pH 2, *yellow* from pH 3 to pH 8.3, and *red* above pH 9.

Estimation of the Titration Acidity of Gastric Contents

The alkali-neutralising power of gastric contents depends on : (i.) *free hydrochloric acid*, and (ii.) *acid salts* and *carboxy compounds* formed or released during digestion. These are sometimes termed *bound hydrochloric acid*. In addition, the abnormal fermentation products, butyric and lactic acid, may be present.

Both forms of acidity (i.) and (ii.) may be estimated consecutively if an appropriate triple-change indicator be used.

Total Titration Acidity

	acid salts + organic acids					
	HCl					
Thymol blue	red	orange	yellow	green	blue	
T.p. indicator	red	orange	yellow	orange	red	
pH	1.4	2	2.8	3	8	9

I. Free Hydrochloric Acid

(1) Fill a burette with 0.1 N sodium hydroxide. Clear the delivery tube of air bubbles. Note burette reading.

(2) Measure carefully by pipette 10 ml. of gastric contents into a flask or large test tube. Add 5 drops of 0.1 per cent. thymol blue or t.p. indicator. A red colour denotes the presence of free hydrochloric acid.

(3) Titrate the mixture, adding the alkali in small quantities, and shaking well after each addition. The end-point is reached when the red changes to orange.

To determine the exact end-point, titrate until the colour matches the yellow tint of a control flask or tube containing 10 ml. of water and 5 drops of indicator.

(4) *Calculation*.—Since 1 ml. of 0.1 N sodium hydroxide is equivalent to 3.65 mg. of hydrochloric acid,

$$x = \frac{n \times 3.65}{100}, \quad \text{where } x = \text{percentage of free HCl in gm. per 100 ml.}$$

n = number of ml. alkali required to neutralise as far as the first change (red-yellow) of the indicator.

II. Acid Salts (and organic acids)

(5) Note the reading of the burette, and continue the titration until the yellow colour of the mixture starts to change to *green* (thymol blue) or to *pink* (t.p. indicator). The change can best be determined by comparing the titration flask with the yellow control flask. As soon as a difference between the two colours can be detected take the final reading of the burette.

(6) *Calculation*.—As before :—

$$x' = \frac{n' \times 3.65}{100}, \quad \text{where } x' = \text{acid salts (and organic acids) expressed as percentage HCl.}$$

n' = number of ml. of alkali required to neutralise as far as the second change of the indicator.

III. Total Acidity

This is the sum of the free acidity and the acid salts (and organic acids). It is obtained by adding x and x' .

Note.—A single estimation merely indicates the presence or absence of an adequate amount of free hydrochloric acid. In clinical practice, the estimation is made serially on samples (2–5 ml.) of contents aspirated from the stomach at fifteen-minute intervals after a meal. Such data show the rate and course of gastric secretion.

Detection of Abnormal Acids in Gastric Contents

Butyric acid, $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$, can be recognised immediately by its characteristic sour smell.

Lactic Acid, $\text{CH}_3 \cdot \text{CH}(\text{OH}) \cdot \text{COOH}$.—(a) *In the absence of free hydrochloric acid*.—Add just sufficient dilute (1 per cent.) ferric chloride to 10 ml. of water to make it faintly yellow. Divide the mixture into two parts. To one tube add 2–3 ml. of the gastric contents (filtered, if necessary), and compare the colour of the two tubes. If lactic acid is present, the colour deepens to greenish-

yellow. Unless this test is checked against the control tube it is unreliable.

(b) *In the Presence of Free Hydrochloric Acid.*—The mixture must be extracted with ether, and the ferric chloride test applied to the extract.

Shake up for a few minutes 5 ml. of filtered gastric juice with 20 ml. of ether, preferably in a separating funnel. Allow the mixture to separate into two layers. Remove 5 ml. of the ether carefully so as to avoid contamination with gastric contents. Add 10 ml. water, and about 10 drops 1 per cent. ferric chloride solution. Shake gently. If the gastric filtrate contained 0.05 per cent. lactic acid the mixture will turn greenish; over 0.1 per cent. produces a distinct yellow.

A fallacy may be introduced into either form of the test by the presence of thiocyanate from swallowed saliva. This gives a red colour with ferric chloride, but, unlike the lactate, it is bleached on addition of a few drops of 5 per cent. mercuric chloride.

DUODENAL DIGESTION

This is brought about by the combined action of three secretions: pancreatic juice, bile, and intestinal juice, or *succus entericus*. Digestive activity is maximal about the second stage of the duodenum, and the process continues throughout the small intestine.

Percentage Composition of Human Pancreatic Juice

Organic Solutes, 0.6–0.7.				Inorganic Solutes, 0.7.			
Trypsinogen	.	.	+	Na ⁺	0.25	Cl ⁻	0.35
Pancreatic erepsin	.	.	+	K ⁺	0.0008	CO ₃ ⁻	+
Amylase	.	.	+	Ca ⁺⁺	+	HSO ₄ ⁻	+
Lipase.	.	.	+	Mg ⁺⁺	+	H ₂ PO ₄ ⁻	+
Lipides	.	.	0.52				

The pH of human pancreatic juice is about 8, and the alkalinity corresponds approximately to a 0.53 per cent., or N/10 solution of sodium carbonate, hence one volume of gastric juice is neutralised by about the same volume of pancreatic juice.

Identification of the Constituents of Pancreatic Juice.—The most characteristic constituent of pancreatic juice is the enzyme trypsin, which is secreted in the inactive form as trypsinogen, and is activated by the enterokinase of the intestinal juice. Trypsin is identified by its proteoclastic action on fibrin in alkaline solution. Three test

tubes are prepared and incubated, as in the method for identifying pepsin in gastric juice (p. 269).

To *a* add 5 ml. pancreatic juice made alkaline with 1 per cent. sodium carbonate.

To *b* add 5 ml. pancreatic juice, acidified with 0.1 N hydrochloric acid.

To *c* (the control tube) add 5 ml. 1 per cent. sodium carbonate.

Incubate and examine, as before. The presence of trypsin, the protein-splitting enzyme that operates in alkaline solution, is shown by the digestion of the protein in tube *a*.

Lipase.—This enzyme can only be found in fresh extracts of the pancreas or juice recently secreted. It is detected by the fat-hydrolysis test previously described in connection with the lipides.

Amylase.—This enzyme is detected by the starch-hydrolysis test described for ptyalin.

BILE

Bile may be obtained in two forms : fistula bile, the fresh secretion of the liver ; and gall-bladder bile, which has been stored and concentrated previous to being poured into the duodenum. The daily secretion of fistula bile is fairly constant, and usually between 600 and 700 ml. in twenty-four hours.

Percentage Composition of Human Bile

Fistula Bile.	Bladder Bile.
Total solids . . . 1.92	Total solids . . . 11.0
Bile salts . . . 0.715	Bile salts . . . 6.0
Pigments . . . 0.15	Pigments . . . 2.0
Cholesterol . . . 0.057	Cholesterol . . . 0.375
Nucleoprotein . . . +	Nucleoprotein . . . +
Mucin . . . +	Mucin . . . +
Inorganic solutes . . 0.7	Inorganic solutes . . 0.8

Significance of the Constituents of Bile.—(i.) **Bile Salts.**—These are the sodium salts of glycocholic acid, $C_{26}H_{43}NO_6$, and taurocholic acid, $C_{26}H_{45}NSO_7$, which are esters of cholic acid.

In the resting state before meals, about 95 per cent. of the total bile salts of the body are collected in the gall bladder.

The bile salts are the active digestive agents in the bile secretion. They have a very bitter taste. They lower surface tension, and

thus promote : (i.) emulsification of food colloids, and (ii.) intestinal absorption of lipides. During the process of absorption, the bile salts act as carriers of the fatty acids, and are reabsorbed into the circulation, and resecreted by the liver, the process resulting in a continuous circulation of bile salts. When administered by the mouth, they act as a powerful natural cholagogue.

In obstructive jaundice, lack of the bile salts in the intestine gives rise to digestive disturbances due to the inability to absorb the hydrolysed fat.

Cholic acid is synthesised and esterified in the liver. Its precursors are obscure, and probably form part of the system whereby cholesterol is manufactured in the organism.

(ii.) **Cholesterol.**—With the possible exception of milk, bile is the only human secretion containing any considerable amount of cholesterol, and it is the chief channel of excretion of the sterol. The range of concentration is very great ; values of from 0.01 to 1.3 per cent. have been obtained for bladder bile, the average being 0.3, corresponding to a daily output of 0.34–0.4 gm. Biliary cholesterol is highly important in connection with the formation of sterol calculi or “gall stones.”

“The liver possesses the power of synthesising both cholesterol and cholic acid, and thus controls the amount of these substances present in the bile. Under normal conditions the amount of cholic acid produced synthetically is probably small, because the bile salts are almost completely reabsorbed from the intestine and return to the liver.

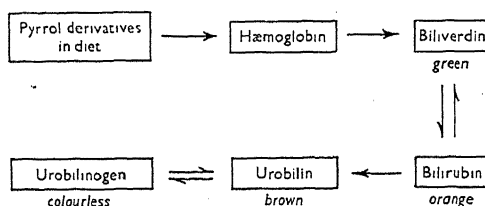
“The production and reabsorption of cholesterol is a much more variable process. The liver probably obtains some of its supply from the diet, also from the breakdown of old blood corpuscles. Reabsorption hardly occurs at all in some animals, while with others it is practically complete. In the intestine, cholesterol frequently undergoes more or less complete reduction to coprosterol, and this appears to be one of the factors limiting reabsorption” (Fox, 1927).

(iii.) **Bile Pigments.**—The orange-red pigment, *bilirubin*, and the green pigment, *biliverdin*, occur in varying proportions in the bile of different animals, and may be accompanied by related pigments. Human bile is golden yellow owing to the presence of bilirubin. Ox bile is green owing to biliverdin and phylloerythrin. Phylloerythrin, as Marchlewski has shown, comes from the chlorophyll of the diet (p. 198). Bilirubin and biliverdin are waste products derived from hæmoglobin, and are formed in the reticulo-endothelial tissue lining the vascular channels of the liver and the spleen, and elsewhere. Extrahepatic pigment is transported to the liver by the blood stream, which has a bilirubin content of 0.1–0.5 mg. per

100 ml. In conditions of obstructive jaundice, bilirubin accumulates in the blood until the renal threshold limit of 2 mg. per 100 ml. is exceeded, when the pigment overflows into the urine.

The conversion of hæmoglobin into bile pigment involves four stages : (i.) hydrolysis into globin and hæmatin ; (ii.) conversion of hæmatin into protoporphyrin by removal of Fe ; (iii.) conversion of protoporphyrin into biliverdin ; and (iv.) reduction of biliverdin to bilirubin. The pigments enter the intestine in the bile, and are not reabsorbed, but undergo bacterial reduction to urobilins which make up the stercobilin pigment that colours the intestinal contents. When there is excessive formation of urobilin, some of it is absorbed, and, in part, reduced to urobilinogen, which is excreted by the kidney. On oxidation, urobilinogen forms urobilin, the brown pigment of the urine.

Inter-relationship of the Bile Pigments



Identification of the Chief Constituents of Bile.—I. Pigments

Note the characteristic orange colour due to bilirubin, and the viscosity due to nucleoprotein and mucin. Fresh bile is slightly alkaline, but its colour is liable to obscure the indicator. For the purposes of the following tests pig's bile is used, previously diluted with water, about 1 : 20 :—

Extract 5–10 ml. of dilute bile with about 3 ml. of ether or toluene. No pigment is removed if the bile be fresh. Acidify the mixture with a couple of drops of concentrated hydrochloric acid, and mix by inversion. The ether or toluene is coloured yellow by the liberated bilirubin.

(1) **Froth Test.**—Shake up some diluted bile in a test tube. The froth that forms has a transient golden-yellow colour. Add 5 to 10 drops of 0.05 per cent. methyl violet. The solution acquires a dark red shade, due to the mixture of the two pigments. Both these simple tests are of use in detecting bile pigment in urine (biliuria).

(2) **Iodine Test** (W. G. Smith).—To 5 ml. dilute bile add about 10 drops of 1 per cent. alcoholic solution (or tincture) of iodine, so

as to form a layer on top. A green ring of *biliverdin* gradually develops between the layers, owing to oxidation of the bilirubin.

(3) **Diazo Test** (van den Bergh).—Add 2 ml. of alcohol to 2 ml. of dilute bile. Mix well, and add 1 ml. of fresh diazo reagent. A red colour develops owing to the presence of bilirubin. Acidify with hydrochloric acid. The colour changes to violet.

The reagent is given in the appendix. In acid solution it is a specific test for bilirubin, but gives no colour with biliverdin. The reaction is the basis of the standard method for estimating bile pigment in blood plasma by comparing the colour with a control containing a known amount of bilirubin. Values are measured in units, each of which represents 1 part of bilirubin in 200,000, or 0.5 mg. per 100 ml. Fistula bile has a value of about 50 units, or 25 mg. per 100 ml.; blood plasma has about 0.2–0.5 units in health, but may exceed ten times this amount in obstructive jaundice, and in toxic conditions leading to increased destruction of erythrocytes.

In the blood, bilirubin circulates in a masked form that only gives an indirect reaction with the diazo reagent. On reaching the liver it is converted into free bilirubin previous to excretion in the bile.

(4) **Turpentine Test**.—Acidify 5 ml. of bile with glacial acetic acid until the precipitated nucleoprotein redissolves. Add 2 ml. of turpentine, shake well, and heat carefully without boiling.

The white turpentine emulsion gradually turns green owing to formation of biliverdin.

II. Bile Acids (Taurocholic and Glycocholic Acid)

(1) **Sulphur Test** (Hay).—Sprinkle some finely powdered sulphur on the surface of 10 ml. of dilute bile in a test tube. The particles gradually penetrate the surface and sink through the liquid owing to the reduction in surface tension due to the bile salts.

Compare with a control experiment carried out by sprinkling sulphur on water, the surface of which is able to support the particles.

(2) **Sugar Test**.—Dissolve a little lactose in not more than 10 drops of undiluted bile. Add excess (10 ml.) of concentrated hydrochloric acid. Mix well. Boil carefully over a small flame. A dull purple colour develops.

In Pettenkofer's original form of the test a trace of sucrose is mixed with concentrated sulphuric acid, and a few drops of bile are added. Under favourable conditions a deep purple colour is produced, but the mixture is very liable to be obscured by charring.

Both tests resemble the thymol test for carbohydrates. The

phenol is replaced by cholic acid, which combines with the furfural set free from the sugar (p. 112).

III. Proteins

When bile is acidified a dense precipitate appears, consisting of *nucleoprotein*, which is soluble in glacial acetic acid, and *mucin*, which is insoluble in the acid. The proportions of these proteins differ in different animals. Ox bile is rich in nucleoprotein, but poor in mucin. By careful precipitation the proteins can be precipitated, filtered off, washed, and redissolved in sodium carbonate solution. They give the tests characteristic of their type (p. 130).

IV. Cholesterol

There is no simple direct test for cholesterol in bile. It can be identified in biliary calculi, or in the residue from the ethereal extraction of bile dried on a water-bath, by the characteristic shape of the crystals and by the sterol colour tests (p. 175).

INTESTINAL JUICE

The mixed secretion of the small glands of the duodenum, jejunum and ileum constitutes the intestinal juice, or *succus entericus*. As obtained from an experimental fistula in the dog, it is an alkaline liquid of pH 8-10, and contains a great variety of enzymes, which complete the hydrolyses begun by the catalysts of the earlier secretions.

Composition of Dog's Intestinal Juice

Organic Solutes, 0·6-0·7 per cent.				Inorganic Solutes, 0·7 per cent.	
Erepsin	+	Maltase	+	NaCl	0·5
Nucleinase	+	Lactase	+	Ca ⁺⁺	+
Nucleotidase	+	Sucrase	+	Mg ⁺⁺	+
Enterokinase	+	Amylase	+	HCO ₃ ⁻	+
Esterase	+				

The intestinal mucosa contains two important endo-enzymes: a *phosphatase*, which phosphorylates the hexoses previous to their absorption, and a *nucleosidase*, which hydrolyses the nucleosides derived from the nucleoproteins (p. 130).

Summary of the Digestive Process

Region.	Secretion.	Reaction.	Enzymes.	Substrates.	End-products.
Mouth	Saliva.	Slightly acid or neutral; pH 5.8-7.0.	Ptyalin (amylase). Maltase.	Starch. Dextrin. Glycogen. Maltose.	Maltose. Glucose.
Stomach	Gastric juice.	Acid; pH 1.7.	Pepsin. Rennin. Lipase.	Proteins. Caseinogen. Fats.	Peptides. Casein. Aliphatic acid + glycerol.
Intestine	Pancreatic juice.	Alkaline; pH 8-10.	Trypsin. Erepsin. Amylase. Maltase. Lipase.	Proteins. Peptides. Starch, etc. Maltose. Fats.	Peptides. Amino acids. Maltose. Glucose. Acid + glycerol.
	Bile.	Alkaline; pH 8.	None.		
	Intestinal juice.	Alkaline; pH 9.	Erepsin. Sucrase. Lactase. Nucleinase. Nucleotidase. Enterokinase.	Peptides. Sucrose. Lactose. Nucleic acids. Nucleotides. Trypsinogen.	Amino acids. Glucose + fructose. Glucose + galactose. Nucleotide. Nucleoside + H_2PO_4 . Trypsin.
Intestinal mucosa.	—	—	Nucleosidase. Phosphatase.	Nucleosides. Hexoses.	Purines + sugar. Hexose phosphate.

INTESTINAL ABSORPTION

The efficiency of the human digestive process in health is such that about 96 per cent. of the total mixed dietary is made available for absorption into the portal and lymphatic systems. Colloids have been converted into diffusible solutes, insoluble fats have been emulsified and saponified, compound saccharides have been changed into utilisable monosaccharides, and foreign proteins have been rendered non-toxic by being hydrolysed into non-specific mixtures of amino acids.

The faculty of absorption resides in the entire alimentary tract, being maximal in the small intestine. Absorption in the mouth and œsophagus is negligible, gastric absorption is almost entirely restricted to alcohol and CO_2 , colonic absorption is chiefly water.

For a nutrient to be absorbed, either it must be water-soluble and of low molecular weight, or must be combined with a carrier such as the bile acids. The first form of absorption is almost indiscriminate; the second form shows high discrimination with regard to certain reactants.

Absorption of Aqueous Solutes.—The chief factors determining

the absorption of water and simple solutes are diffusion and osmosis. The absorption of a solute follows its diffusion gradient, or relative concentrations in the two media. When the concentration is greater in the intestine than in the blood it moves from the intestine. Water is absorbed as soon as the solution in the intestine has a lower osmotic pressure than that of the blood.

An isotonic solution of glucose or sodium chloride loses its solute so rapidly that it becomes hypotonic, and absorption of the solvent occurs. An isotonic solution of a non-diffusible solute, such as magnesium sulphate, or a sugar of low diffusibility, such as xylose, remains unchanged for a long time in the intestine; while a hypertonic solution of these compounds can act as a cathartic by abstracting water from the blood, and thus diluting the intestinal contents. The biological hexoses in particular, are rapidly absorbed owing to the presence of phosphatase in the intestinal mucosa, which transforms them into phosphoric esters, and thereby increases the diffusion gradient.

Proteins are absorbed in the form of amino acids and also, to an uncertain extent, as simpler polypeptides (London and Kotschneff, 1928-34). Absorption of intact protein by a defective mucosa is responsible for allergy, or hypersensitiveness to certain foodstuffs, such as egg-white and milk protein.

In the large intestine, where most of the food solutes have been removed, water absorption is chiefly a filtration due to hydrostatic pressure (Verzár and McDougall, 1936).

Absorption of Lipides.—In order to be absorbed, simple and complex lipides must first undergo hydrolysis to aliphatic acids. These acids then combine with the bile salts to form water-soluble, diffusible complexes, which pass into the intestinal mucosa, where they interact with glycerophosphoric acid to regenerate the neutral fat, and thus enter the lymphatics. Hence, three factors are concerned in fat absorption: (i.) the enzyme lipase and its activators; (ii.) the bile salts, which act as carriers; (iii.) the phosphorylation mechanism in the mucosa, which in turn, requires vitamin B₂ (p. 257) and the hormone of the adrenal cortex (p. 415), as demonstrated by Verzár. This elaborate mechanism endows the organism with considerable power of discrimination in the absorption of lipides and lipoids, as shown by the preferential absorption of carotene.

Man absorbs cholesterol more readily than the related sterols, but shows no preference for any one of the carotinoids. The cow absorbs carotene and excludes xanthophyll, although the latter predominates in herbage. The absorption of carotinoids and vitamin A is greatly facilitated by the presence of fats in the dietary.

GENERAL REFERENCES

- ALVAREZ, W. (1924), "Intestinal auto-intoxication." *Physiol. Rev.*, **4**, 352.
- ALVAREZ, W. (1928), "Mechanism of the Digestive Tract." New York.
- BENNETT, I. (1925), "The Stomach and Upper Alimentary Canal in Health and Disease." London.
- CARLSON, A. J. (1923), "Secretion of gastric juice." *Physiol. Rev.*, **3**, 1.
- IHRE, B. J. E. (1939), "Human Gastric Secretion." London.
- IVY, A. C. (1934), "Physiology of the gall bladder." *Physiol. Rev.*, **14**, 1.
- MAGEE, H. E. (1930), "The role of the small intestine in nutrition." *Physiol. Rev.*, **10**, 473.
- SOBOTKA, H. (1937), "Physiological Chemistry of the Bile." London.
- VERZÁR, F., and H. J. McDOUGALL (1936), "Absorption from the Intestine." London.
- YOUNGE, C. M. (1937), "Evolution and adaptation in the digestive system of the metazoa." *Biolog. Rev.*, **12**, 87.

CHAPTER XVI

INTERMEDIATE METABOLISM : CARBOHYDRATES

INTERMEDIATE metabolism comprises the history of the biochemical compounds within the organism, starting with the changes undergone by the foodstuffs after absorption into the blood stream. In theory, each molecular species has its own independent career from the time it passes into the portal blood or lymphatic system, but observation shows that a physiological co-ordination, or *homeostasis*, exists controlling the chemical mechanisms of intermediate metabolism.

While the food materials remain in the alimentary tract they are physiologically outside the organism ; it is only after absorption they come into direct contact with the body tissues and participate in the operations of life.

CARBOHYDRATE METABOLISM

The carbohydrates enter the animal in the form of *monosaccharides* ; as pentoses, $C_5H_{10}O_5$, derived from nucleoproteins and pentosans, and as the three *hexoses*, $C_6H_{12}O_6$, glucose, fructose, and galactose, derived from the starches and sugars of the dietary.

Pentose metabolism is obscure. The organism has a low tolerance for these sugars, and they readily appear in the urine after ingestion of fruits and vegetable gums (alimentary pentosuria). At the same time they may be synthesised to meet the demands of the growing tissues for nucleoproteins ; and in the condition of congenital pentosuria they are excreted continuously, irrespective of the pentoses derived from the diet.

Hexose Absorption.—The relative speed of sugar absorption from the intestine is in the order : galactose > glucose > fructose ; the concentration of sugar affects the duration but not the rate of absorption, showing that it is not a simple diffusion process.

The hexoses on entering the cells of the intestinal wall are converted into the corresponding phosphoric esters, and by this means the diffusion gradient is kept favourable for sugar absorption. Pentoses, disaccharides and hexoses other than D-glucose, D-fructose, D-galactose and D-mannose are not phosphorylated by the

intestinal enzyme system, and their rate of absorption is relatively slow, and is regulated by simple diffusion.

By means of iodoacetic acid or the glycoside *phloridzin*, it is possible to inhibit the phosphorylating mechanism in the intestinal wall, and when this is done, the preferential absorption of hexoses ceases, and they diffuse in a manner similar to the other saccharides.

Hepatic Function.—All the glucose absorbed in excess of the immediate requirements of the tissues travels in company with the fructose and galactose of the diet to the liver, where they are converted into glycogen, the storage polysaccharide of the body. This process of polymerisation involves the interconversion of the three hexoses into a common unit, and when glycogen is broken down again the product is glucose. Thus the liver both transforms and stores carbohydrate.

The glycogen content of the liver is variable, and the average value for the human adult is about 3 per cent. of the fresh tissue. A meal rich in carbohydrates may raise the hepatic glycogen of a dog or a rabbit up to 7 per cent., but this level is not maintained during daily activity.

The total amount of glycogen in the adult human body is assessed at the average value of 200 gm., approximately half being in the muscles and half in the liver. Muscle glycogen is part of the contraction mechanism, and yields lactic acid when broken down; hepatic glycogen is the storage polysaccharide of the body, and yields glucose. Muscle glycogenolysis is part of a complex series of events (the Parnas cycle); hepatic glycogenolysis is brought about by an endo-enzyme, *glycogenase*, working in conjunction with extra-hepatic factors.

<i>Glycogen</i>	<i>Glucose</i>
Hepatic glycogen synthesis is promoted by :—	Hepatic glycogen resolution is promoted by :—
(1) High blood sugar level.	(1) Low blood sugar level.
(2) Insulin.	(2) Adrenalin.
	(3) Asphyxia.
	(4) Low temperature.

The Blood Sugar.—The venous or capillary concentration of sugar in the post-absorptive stage (twelve to sixteen hours after a meal) is usually within the limits 70–120 mg. per 100 ml. Prolonged starvation may reduce the level to half its normal value. Immediately after ingestion of glucose (50–100 gm.) the blood sugar level rises above the fasting value in both venous and capillary blood, being lower in the former owing to the continual transfer of sugar from the blood to the tissues. The arterial maximum is reached in

twenty to forty minutes, about the region 170–220 mg. per 100 ml. The venous maximum is 30–70 mg. lower, and is reached about the same time.

The rise in blood sugar concentration is not proportional to the amount of sugar administered. Doses above 50 gm. stimulate a utilisation mechanism whereby glucose is removed from the circulating blood.

The Glucose Threshold.—When the glucose concentration in the blood rises to a critical maximum, between 114 and 216 mg. per 100 ml. in health, sugar is excreted in the urine, the condition being one of hyperglycæmic glycosuria. The maximum is termed the “rising glucose threshold,” the implication being that as soon as the sugar concentration exceeds a certain limiting value it overflows into the urine.

Glucose is an essential metabolite, and has a high renal threshold value. It passes through the glomeruli, along with the other filtrates from the plasma, and is re-absorbed by the convoluted tubules. Renal absorption of glucose is so efficient that only traces of the sugar appear in normal urine. The absorption is determined by the formation of phosphoric esters in the tubule cells, and is comparable to the absorption of glucose from the intestine. Foreign sugars, such as pentoses, which do not undergo esterification by phosphatase, pass through the tubule into the urine, where they contribute to the reducing substances normally present. If the blood glucose exceeds a limiting value, or if the renal absorption mechanism is defective, the filtrate is not freed from glucose, and a condition of true *glycosuria* results. By injection of phloridzin in doses of above 0·5 gm., it is possible completely to inhibit the renal phosphatase. In this condition of *phloridzin diabetes*, the renal threshold for glucose is lowered almost to zero, and glycosuria results, until the animal reaches a state of severe hypoglycæmia, owing to depletion of its carbohydrate reserves.

Glucose tolerance expresses either (a) the total amount of carbohydrate that can be consumed daily without evoking glycosuria, or, more exactly (b) the dosage of glucose necessary to raise the blood sugar above the renal threshold. Alimentary glycosuria from carbohydrate over-feeding is rare in health, as the resources of the organism for carbohydrate storage and conversion are considerable.

In some pathological conditions, notably *diabetes mellitus*, glucose tolerance is low, and excess of carbohydrate in the diet readily evokes glycosuria.

Hypoglycæmia.—When the glucose content of the blood falls below a critical value, 0·07–0·03 per cent. in man, a characteristic hypoglycæmic syndrome sets in, the signs and symptoms of which

are : (i.) extreme hunger, (ii.) fatigue and prostration, (iii.) motiveless anxiety, (iv.) tremors, (v.) vaso-motor unbalance with flushing or pallor, (vi.) delirium, coma, loss of deep reflexes. Hypoglycæmia has little effect on the contraction or irritability of the denervated muscle, and it is inferred that the motor disturbances arise centrally from glucose starvation of the nervous system. Hypoglycæmia may be evoked by injection of insulin, and abolished by injection of (i.) glucose, (ii.) adrenaline, which promotes hepatic glycogenolysis, and by (iii.) anterior pituitary extract, which antagonises insulin.

Experimental removal of the liver results in a rapid fall in blood sugar. Hepatectomised dogs require glucose to be infused at 0.25 gm. per kg. body weight per hour if hypoglycæmia is to be averted. This indicates that the liver of a 10 kg. dog during carbohydrate starvation is secreting about 64 gm. of glucose in twenty-four hours; and, if the human body be comparable in efficiency, man's liver is producing up to 400 gm. of glucose *per diem*, an amount but little below the carbohydrate content of the ordinary diet.

Sources of Blood Sugar

(1) *Carbohydrates*.—While it is believed that all, or almost all, of the blood sugar normally comes from hepatic glycogen, substances other than the simple hexoses can contribute either to glycogen or to sugar formation. The simplest of these are (+)-lactic acid (sarcolactic acid) and glycerol. Lactic acid is the characteristic end-product in muscle metabolism, and is produced in brain and other tissues during glycolysis. Glycerol is a constituent of all fats and complex lipides, and is liberated during digestion. The relative efficiency of these substrates has been computed by the Coris, who measured the glycogen increase in the livers of rats, previously starved for twenty-four to forty-eight hours, until the hepatic glycogen had fallen to 0.1–0.2 per cent.

Food Material.	Absorption Time.	Amount absorbed per 100-gm. Body Weight.	Liver Glycogen.
Glucose . .	4 hours.	1.06 gm.	5.3 per cent.
Fructose . .	4 „	0.54 „	5.7 „
Galactose . .	4 „	1.10 „	1.2 „
(+)-Lactic acid . .	3 „	0.11 „	1.2 „
Glycerol . .	4 „	—	2.4 „

(2) *Proteins*.—Animals on a carbohydrate-free diet continue to store liver glycogen, showing that it can be obtained from sources

other than food saccharides. Of these, the most obvious are the surplus amino acid residues after removal of the nitrogen. In diabetic animals unable to utilise sugar, the food or tissue proteins are diverted to the manufacture of the glucose which appears in the urine. Under these conditions the sugar : nitrogen output in the urine, or D/N ratio, is about 2.8 : 1. In animals unable to retain sugar owing to phloridzin injections, the D/N ratio in the urine is as high as 3.6 : 1. Since 100 gm. of food protein yield about 16 gm. of urinary nitrogen, the value of the D/N ratio indicates that, in favourable circumstances, up to 58 per cent. of protein can be transformed into glycogen or glucose. By individual feeding experiments it has been shown that the glucogenic amino acids are arginine, proline, hydroxyproline, cystine, serine, alanine, glycine, glutamic acid, hydroxyglutamic acid and aspartic acid (Lusk, 1928).

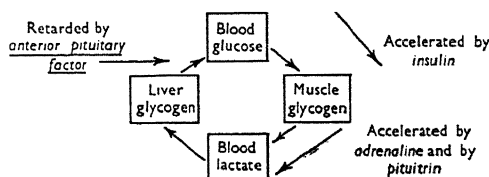
(3) *Fats*.—Addition of fat to the diet of a depancreatized or otherwise diabetic animal neither increases liver glycogen nor sugar excretion, and it was formerly assumed that carbohydrate cannot arise from fat in the animal body, apart from 10 per cent. of the molecule, which is released as glycerol. In pancreatic diabetes, however, the respiratory quotient may fall as low as 0.7, which indicates that fat alone is being oxidised.

The utilisation of fat in the absence of sufficient carbohydrate presents difficulties to the animal organism, and when the fat content of the dietary exceeds a limiting proportion, harmful metabolites, β -hydroxy butyric acid, acetoacetic acid and acetone, are formed, and tend to induce a ketosis. In the picturesque language of the last century, "fats burn in the fire of the carbohydrates, and the smaller the fire the greater the smoke." This is not entirely true. Carbohydrate oxidation is chiefly extra-hepatic, whereas ketone formation is hepatic; hence, all, or most of the aliphatic acids are not necessarily dependent on sugar for their combustion. Young has calculated that, during complete starvation, only about half of the sugar secreted daily by the liver can be accounted for by protein break-down, glycerol, and all possible carbohydrate sources other than the aliphatic acids. He suggests that "the liver of the fasting animal, whether the pancreas is present or not, is converting fatty acids to sugar at the maximum rate possible, so that addition of dietary fat cannot further stimulate carbohydrate formation. . . . If exogenous carbohydrate is supplied then the hepatic non-carbohydrate sources of sugar can be spared to a considerable extent" (1936).

The Glucose-lactate Cycle.—Lactate is a constant constituent of human blood, the range being 5–20 mg. per 100 ml. in the resting

subject. Moderate exercise may raise the value to 40 or 50 mg., while violent exercise has raised it as high as 200 mg. (A. V. Hill, 1924).

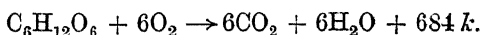
A fraction of this lactate is excreted by the urine, a fraction may undergo oxidation in the tissues, but the bulk of it, as Cori and Cori have shown (1929), is rebuilt into glycogen in the liver. Thus, a sugar molecule can traverse a complete cycle in the organism.



The glucose-lactate cycle. (Cori and Cori.)

The Heat Value and Respiratory Quotient of Carbohydrates.—

Each gram molecule of hexose completely oxidised in the body requires six molecules of oxygen, and forms six molecules of carbon dioxide, the process being accompanied by the liberation of 684 kilocalories of heat.



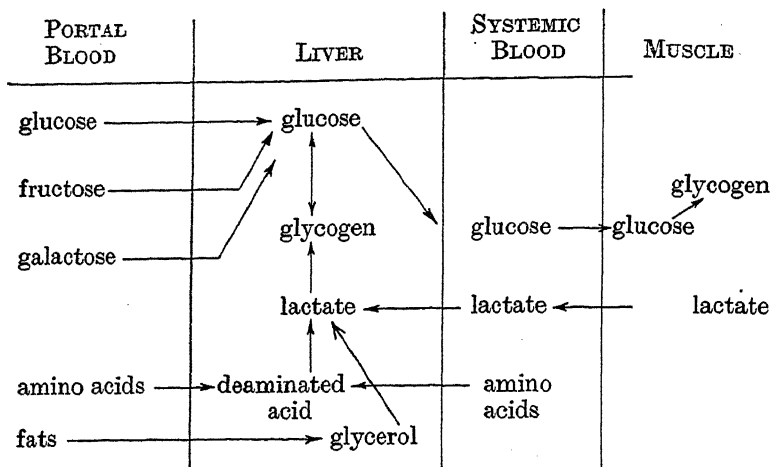
Expressed otherwise, the heat value of a carbohydrate is approximately 4.0 kilocalories per gram, the value being slightly higher for the polysaccharides, which are less hydrated. Also, since the volume of oxygen consumed equals the volume of carbon dioxide produced, the respiratory quotient is 1 where *R.Q.* (the quotient) is expressed as CO_2/O_2 .

When the respiratory quotient of an organism or a tissue is unity, the inference is drawn that carbohydrate, or possibly lactate is the substance oxidised.

Oxygen and Carbohydrate consumed in Walking One Mile at Different Speeds (A. V. Hill, 1933)

Speed, in Miles an Hour.	Heat Liberated, in Kilocalories.	Rise of Body Temperature.	Oxygen consumed, litres.	Sugar Oxidised, grams.
2	30	0.5° C.	6	8
3.5	60	1	12	16
5	115	2	23	31

Carbohydrate Circulation in the Animal Body



CARBOHYDRATE UTILISATION

Carbohydrates, especially glucose, provide an important source of energy for living cells. This energy is obtained in two different ways: (i.) In the absence of free oxygen, by fermentation, or glycolytic degradation of the sugar into simpler compounds with a lower energy value, and (ii.) in the presence of oxygen, by respiration, or oxidation.

(i.) **Anaerobic glycolysis**, or fermentation, appears to be a primitive endowment of all cells, and is characteristic of the life of lower plants, notably yeasts and bacteria. The process is uneconomical in that the end-products are lactate, alcohol or similar combustible compounds, the energy of which is unavailable for the organism. In higher animals, anaerobic glycolysis is restricted almost entirely to the muscular system, where it forms part of the highly complex and specialised mechanism of contraction. Malignant tissues have lost most of their ability to oxidise carbohydrate, and they obtain energy by anaerobic glycolysis, which enables them to grow rapidly in circumstances of restricted oxygen supply.

The Pasteur Effect.—Fermentation is characterised by a high rate of carbohydrate destruction and an accumulation of incompletely oxidised end-products. It is antagonised by free oxygen, which preserves the carbohydrates of the cell from extravagant wastage, and restricts the products of anaerobic metabolism. This inhibition of fermentation by oxygen is termed, the Pasteur effect,

after its discoverer, who showed that the ratio, *weight of sugar destroyed to weight of yeast formed*, during alcoholic fermentation, varied from 176 : 1 (in complete absence of air) to 4 : 1 (in presence of excess of oxygen).

(ii.) **Aerobic oxidation**, or respiration, takes place in presence of free oxygen, and results in the formation of the completely oxidised end-products carbon dioxide and water. Respiration is a fundamental property of all active tissues and is found in all organisms, with the notable exception of the obligatory anaerobes, which fail to grow when the oxygen-content of their environment has reached a value sufficient to inhibit completely their anaerobic metabolism.

(iii.) **Aerobic glycolysis**, or fermentation in presence of oxygen, is displayed by a few fungi, notably brewer's yeast. It does not occur in the majority of animal cells, apart from the production of small amounts of lactate by tissues with an exceptional ability for utilising sugar, such as brain cortex, mammalian retina, and embryonic structures. Cyanide and other respiratory poisons may induce or reveal aerobic glycolysis in tissues by inhibiting the entry of free oxygen into the respiration process.

In the animal, carbohydrate traffic is dominated by the activities of five specialised tissues : (1) skeletal muscle, (2) cardiac muscle, (3) brain cortex, (4) kidney, and (5) liver. Three of these require individual consideration.

1. Skeletal Muscle

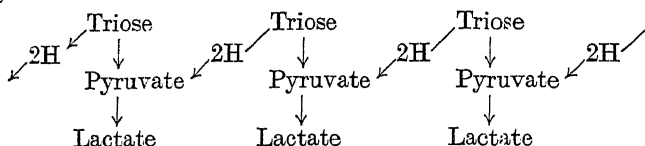
Muscle is an apparatus for the rapid conversion of chemical energy into heat and mechanical work. As regards rate and continuity, this proceeds almost uniformly in cardiac and unstriated muscle, but striated skeletal muscle is under voluntary control and subject to abrupt and occasional demands from the organism. Skeletal muscle, in consequence, requires a store of energy that can be released rapidly under anaerobic conditions, and replenished under aerobic conditions in the resting tissue.

The **contraction process** is accompanied by two physical events capable of exact measurement : heat liberation and mechanical work. The process is anaerobic in that it can continue for some considerable time in absence of oxygen, during which period glycogen is converted into lactate, the characteristic end-product of muscle activity.

In outline the change involves four stages in degradation : (a) glycogen to hexose, (b) hexose to two molecules of a C_3 sugar, or triose, (c) oxidation of triose to pyruvate, and (d) reduction of pyruvate to lactate. The energy required for the reaction is trans-

phosphate and adenosine triphosphate, and is catalysed by a system of at least ten enzymes.

The process forms a cyclic reaction chain in which pyruvate is reduced to lactate by accepting two atoms of hydrogen from a triose, which thereby becomes dehydrogenated to another molecule of pyruvate.



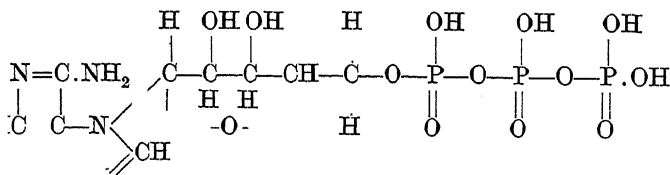
In order to react, the triose and the pyruvate are activated by specific dehydrogenases (p. 328), and intermediate phosphoric esters are formed. Three interlinked chemical systems are operated :

- (1) Adenosine triphosphate \longleftrightarrow adenylic acid + $2\text{H}_3\text{PO}_4$.
- (2) Creatine phosphate \longleftrightarrow creatine + H_3PO_4 .
- (3) Glycogen \longleftrightarrow hexose monophosphate \longleftrightarrow lactic acid.

The first two systems are independent of oxidation ; the third is semi-anaerobic in that glycogen break-down (glycogenolysis) proceeds in absence of oxygen, whereas glycogen formation (glycogenesis) requires oxygen, and takes place during the recovery stage in muscle.

(1) **The Adenosine Triphosphate System.**—Fresh mammalian muscle contains about 0.01–0.02 per cent. of adenosine triphosphate, or adenylyl pyrophosphate, a diphosphoric ester of adenylic acid which is a nucleotide assembled from adenine, D-ribose and phosphoric acid (p. 349).

At the outset of contraction, adenosine triphosphate releases two molecules of free phosphoric acid, which are primary effectors in the contraction process.



Adenosine Triphosphate
(adenyl pyrophosphate).

Adenosine triphosphate is a component of co-dehydrogenase II (p. 259).

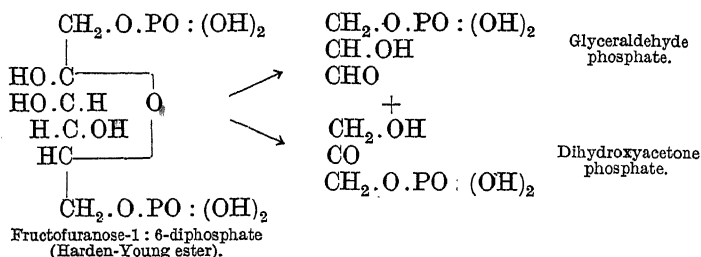
(2) **The Creatine Phosphate System.**—Fresh muscle contains 0.5–0.6 per cent. of creatine, present as a non-diffusible phosphate

The rate of acid formation is greatly increased by contraction under anaerobic conditions, the amount of acid formed being related to the work done by the muscle.

These changes were first elucidated by Hopkins and Fletcher, who taught subsequent workers "to respect the biological qualities" of living tissues. By disintegrating the fresh muscle in ice-cold alcohol they were able to obtain extracts unchanged by catalysis during the process of analytical treatment.

Stage I.—Glycogen is phosphorylated and resolved into sugar units, $\text{glycogen} + \text{H}_3\text{PO}_4 \rightarrow \text{glucose-1-phosphate}$ (Cori ester, p. 100). *Glucose-1-phosphate* is transformed to *glucose-6-phosphate*, and phosphorylated to a hexose diphosphate, *fructose diphosphate*, the Harden-Young ester (p. 107).

Stage II.—Fructose diphosphate splits into two molecules of triose phosphate: *dihydroxyacetone phosphate* and *glyceraldehyde phosphate*.

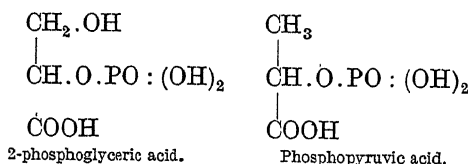


Muscle extracts transform glycogen into lactic acid much more rapidly than they transform glucose into lactic acid, from which it is concluded that the polysaccharide is phosphorylated directly, and not first depolymerised to a hexose.

Stage III.—Two molecules of triose phosphate interact with two molecules of pyruvic acid to form lactic acid and 2-phosphoglyceric acid (α -phosphoglyceric acid).

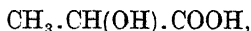
The oxidation of the triose phosphate to phosphoglycerate is catalysed by a dehydrogenase working in conjunction with co-enzyme and adenylic acid.

Stage IV.—2-phosphoglyceric acid is converted to phosphopyruvic acid.



Stage V.—Phosphopyruvic acid reacts with adenylic acid to form adenosine triphosphate and pyruvic acid, $\text{CH}_3\text{CO}\cdot\text{COOH}$.

Stage VI.—Pyruvic acid is reduced to lactic acid,



by the triose phosphate formed in Stage II.

These changes constitute the Parnas cycle of muscle metabolism as revised by later workers, and are partially retraversed during the recovery process. They have been elucidated by comparison with similar changes taking place when sugar is fermented by yeast.

Glycogenolysis Inhibition.—Dialysis of muscle extracts deprives them of glycogenolytic power owing to removal of (i.) magnesium, which activates the muscle phosphatase concerned in phosphorylation of the carbohydrates; (ii.) adenosine triphosphate, the phosphate-carrying co-enzyme of the system. Active inhibition can be brought about by (i.) iodoacetic acid, which poisons the triose phosphate dehydrogenase, and by (ii.) sodium fluoride, which stops the resolution of the hexose diphosphate in Stage II. By a selective use of these specific inhibitors, the intermediate products of glycogenolysis have been concentrated and identified.

The Recovery Process.—A resting skeletal[®] muscle consumes about 6 cubic mm. of oxygen per gm. of dry weight per hour. During active contraction the oxygen consumption rises to 40, or more. This additional oxygen is not required in the contraction, and must be concerned with the recovery process, during which lactic acid disappears and glycogen is regenerated. The respiratory quotient is unity during recovery from the effects of stimulation, which indicates that either carbohydrate or lactic acid is being oxidised to provide energy. Formerly, it was believed that under favourable conditions about one-fifth of the liberated lactic acid was oxidised in the muscle, the remainder being reconverted into glycogen. This appears to be correct for frog muscle. Contraction in higher animals is accompanied by an increase in blood lactate, showing that this metabolite has been diverted into the general circulation to participate in the glucose-lactate cycle.

2. Cardiac Muscle

Heart muscle differs from skeletal muscle in structure and in function. During the lifetime of the animal the heart never rests. Its activity is almost constant, and its recovery period is limited to the refractory phase between each beat. Its creatine content is only 0.2–0.3 per cent., or about half that of skeletal muscle.

Heart muscle contains 0.1–0.2 per cent. of glycogen, which

under anaerobic conditions is transformed into lactate, showing that the heart possesses a metabolic equipment resembling that of skeletal muscle. Apart from this, the two tissues differ in fundamental respects. The heart has a respiratory quotient between 0.8 and 0.9, which signifies that either protein or a mixture of fat and carbohydrate are the food materials oxidised. Under the aerobic conditions of normal working, the glycogen content of the heart appears to be constant, and remains constant as long as the heart is supplied with glucose.

This is also observed if the glycogen-lactate cycle be stopped by iodoacetic acid. Under anaerobic conditions, such as those of asphyxia, the heart can continue to beat provided the lactic acid is neutralised as rapidly as it is formed. Unlike skeletal muscle, the heart is very sensitive to lactic acid, and responds immediately by a protective dilatation of the coronary arteries. Unless the perfusion fluid be kept decidedly alkaline (pH 8.0), the oxygen-starved heart stops beating within a few minutes, owing to acid inhibition. When the perfusion fluid is adequately buffered the heart can continue to beat under anaerobic conditions, its source of energy being obtainable from glucose, probably by way of neutral lactate.

These observations show that the heart is endowed with :—

(i.) An aerobic metabolism working under normal conditions, and probably involving an adenine triphosphate + phosphagen mechanism similar to that in skeletal muscle, but regenerated by a different metabolic cycle.

(ii.) An anaerobic metabolism similar to the Parnas cycle, which comes into operation in conditions of oxygen deficiency, and can continue to work as long as the pH of the blood is kept on the alkaline side of 7.5.

3. Brain Cortex

Man's body has two wardens. The autonomic nervous system and its associated endocrine glands determine the unconscious life and personality; the cerebral cortex and its sensory and motor tracts form the apparatus of intellectual expression. Both structures are composed of nerve cells and their extensions and supporting tissues.

The study of the metabolism of nervous tissue is confronted by a problem that is unique and apparently of insuperable difficulty.

In every other active tissue and organ of the body, metabolism and function can be correlated. But the brain, silent and motionless, traffics with the imponderable. That the brain requires an abundance of oxygen is inferred from the elaborate arterial supply,

and the fact that an acute anoxæmia may be sufficient to bring about loss of consciousness in less than a minute. Indeed, as Barcroft has observed, it is strange that the most important tissue in the body should have no oxygen reserves. By a comparison of the O_2 and CO_2 content of the blood entering and leaving the skull cavity, it has been estimated that the oxygen consumption of the dog's brain is approximately 0.14 ml. O_2 per gram of fresh tissue, per minute, a value much higher than that calculated from the oxygen consumption of cortical tissue in the manometer.

That the brain requires glucose for its maintenance is suggested by the disturbances of the central nervous system—*anxiety*, *confusion*, *delirium* and *convulsions*—that constitute the hypoglycæmic syndrome evoked by insulin, and now being invoked, empirically, for the treatment of schizophrenia (*dementia præcox*). Under experimental conditions, brain tissue suspended in saline is able to oxidise a variety of substrates, including glucose, mannose, fructose, glutamate, succinate, glycerophosphate, pyruvate and lactate, but of all these, only glucose and mannose are able to relieve immediately the signs and symptoms of hypoglycæmia, when injected into the depleted animal. For fructose to be effective it requires to be converted into its isomers by the liver. These results lead to the conclusion that glucose is the usual and continual food material of the cortex.

Under aerobic conditions, glucose oxidation by the cortex produces very little lactic acid. Under anaerobic conditions glycolysis sets in, and lactic acid is produced with great rapidity. Brain cortex shows a typical Pasteur effect, in that the quantity of glucose degraded anaerobically is relatively greater than the quantity oxidised under aerobic conditions.

Kendal Dixon (1937) has shown that the addition of potassium salts in high concentration (M/10) to the aerobic system results in a considerable rise in oxygen consumption and a great increase in lactate formation. This reversal of the Pasteur effect is ascribed to an alteration in cell permeability by the K^+ ions.

According to E. Holmes, lactic acid is an intermediate product in both anaerobic and aerobic glycolysis by cortex tissue, the difference being that under aerobic conditions the lactate is oxidised to simpler products, and its concentration can never rise above a value determined by the concentration of the glucose substrate.

Avitaminosis B₁.—The acute signs of vitamin B_1 deficiency in birds, *opisthotonus* (retraction of the head), failure of vision, loss of temperature control, convulsions, have been traced to the accumulation of lactate and pyruvate in the lower part of the brain (Peters, 1936).

Lactate is converted to pyruvate by lactate dehydrogenase, and the pyruvate subsequently undergoes oxidation to simpler products.

Vitamin B₁ provides the co-enzyme necessary for the pyruvate oxidation. Avitaminous brain tissue has a lowered rate of oxygen consumption, which Peters has shown may be restored to the normal value by addition of vitamin B₁.

Summary of the History of Muscle Biochemistry

1903.—Buchner obtained from yeast a cell-free extract, *zymase*, capable of catalysing the breakdown of glucose to alcohol and carbon dioxide.

1906.—Harden and Young showed that *zymase* required phosphate and a co-enzyme, *co-zymase*, in order to act.

1912.—Harden obtained hexosephosphates from fermenting sugars, and showed that phosphorylation is the first stage in zymolysis.

1912.—Embden obtained a cell-free extract from muscle capable of forming lactic acid from hexosediphosphate, *lactacidogen*. Later, he found that normal muscle contains no hexosediphosphate, but only a monophosphate, which is subsequently converted into diphosphate.

1913.—Dakin and Dudley, and Neuberg, independently discovered *glyoxalase*, a widely-distributed tissue enzyme capable of converting methyl glyoxal (or other ketonic aldehydes) into lactic acid (or a corresponding hydroxy acid). Hence, it was suggested that methyl glyoxal is the immediate precursor of lactic acid in muscle metabolism.

1926.—Phosphocreatine discovered in muscle by the Eggletons.

1926.—Meyerhof obtained *glycogenase* from muscle, and showed that it required phosphate and a co-enzyme in order to be able to catalyse the conversion of glycogen to lactic acid.

1928.—Adenosine triphosphate discovered in muscle by Lohmann.

1932.—Lohmann showed that *glyoxalase* requires a co-enzyme, namely glutathione. However, if muscle extract is freed from glutathione it can still transform glycogen, but not methyl glyoxal to lactic acid. This transformation requires the addition of Mg ions and adenosine triphosphate, which were removed by the dialysis. If only glutathione be added, then methyl glyoxal, but not glycogen, is transformed into lactic acid. Hence, it was concluded that the main path for the conversion of lactacidogens to lactic acid is not by way of methyl glyoxal.

1933.—Embden showed that fluoride inhibits formation of lactic acid from glycogen in muscle tissue, and leads to an accumulation

of *phosphoglyceric acid*, derived from the hexosediphosphate. Phosphoglyceric acid is readily transformed to pyruvic and phosphoric acids by muscle enzymes in the absence of fluoride. From these data Embden suggested a cycle, involving four stages: (i.) resolution of hexosediphosphate into two molecules of *triose phosphate* (glyceraldehyde phosphate and dihydroxyacetone phosphate); (ii.) dismutation of the triose phosphates into glycerophosphate and phosphoglyceric acid; (iii.) resolution of phosphoglyceric acid into pyruvic and phosphoric acids; (iv.) interaction between pyruvic and glycerophosphoric acids to form lactic acid and a triose phosphate (glyceraldehyde phosphate), which rejoins the cycle at Stage (ii.).

1933.—Meyerhof confirmed and extended Embden's observations by showing that pyruvate accumulates in muscle poisoned by sulphite, and is derived from the hexosediphosphate, and not, as was suggested, from lactic acid by dehydrogenation. Three muscle enzymes were separated: *aldolase*, which catalyses the conversion of hexosediphosphate into triosephosphates; *phosphoglyceromutase*, and *enolase*, which catalyse the chain reaction whereby phosphoglyceric acid gives rise to pyruvic acid.

1934.—Lundsgaard showed that muscle poisoned by iodoacetic acid is able to contract for a short time in an atmosphere of nitrogen without producing lactic acid, the energy being derived from the breakdown of phosphagen (phosphocreatine). Hexosediphosphate and triosephosphates accumulate, but are unable to undergo further change.

1934.—Parnas showed that in muscle extracts glycogen reacts with inorganic phosphate to form a hexosemonophosphate, now identified as Cori's ester (p. 100), from which the diphosphate is subsequently derived.

1934.—Jost showed that anaerobic breakdown of carbohydrate to lactic acid in kidney tissue proceeds on the same lines as in muscle.

A similar type of cycle was demonstrated in brain (Euler, 1936), and in heart muscle (Ochoa, 1937).

Sugar Fermentation by Yeast

Natural sugars are liable to attack by yeasts and bacteria, and undergo either aerobic oxidation or anaerobic fermentation, according to circumstances, which led Pasteur to describe fermentation as "life without air." Brewer's yeast (a culture yeast of the species *Saccharomyces cerevisiae*) and some facultatively anaerobic bacteria (the lactic and propionic bacilli), however, are able to ferment

sugars when oxygen is present, and for this reason are employed industrially in the production of alcohol and lactic acid.

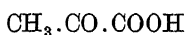
The term *fermentation* (L. *fervere*, to boil) was originally applied to the breakdown of sugar by yeast, on account of the liberation of gaseous carbon dioxide, which caused the mixture to froth. Subsequently, the term was applied to any non-putrefactive change brought about by the growth of living organisms. Other common fermentations include: the *lactic fermentation*, or souring of milk, in which lactic acid is formed from lactose; the *acetic fermentation*, or souring of wine, owing to conversion of ethyl alcohol into acetic acid; and the *ammoniacal fermentation* of urine, in which urea is converted into ammonium carbonate.

Alcoholic fermentation of sugar is represented by the outline equation,

in which the monosaccharide represents one of the four fermentable hexoses, D-glucose, D-mannose, D-fructose and D-galactose. The last of these is only attacked by yeasts previously conditioned by being cultivated in a medium containing galactose.

Sugar fermentation follows the same path as muscle glycolysis in that a chain of intermediate phosphate esters is formed; but when the pyruvate stage is reached, the enzyme *pyruvic carboxylase*, which is present in yeast but not in muscle, decarboxylates the pyruvic acid to *acetaldehyde* and *carbon dioxide*, a characteristic end-product of the fermentation.

The acetaldehyde is subsequently reduced to ethyl alcohol.



Pyruvic acid



Factors in Sugar Fermentation.—(1) *Zymase*.—Cell-free extracts from yeast are able to ferment sugars in absence of living organisms. This property was ascribed to an enzyme complex *zymase*, present in the extract.

Harden and Young later showed that zymase solutions were inactivated by dialysis owing to the loss of two indispensable coactants, both of which were heat-stable. The first of these proved to be inorganic phosphate, the second was an organic compound, which Harden called "the co-ferment of alcoholic fermentation." This organic factor, renamed *co-zymase*, or co-enzyme I, was identified by Euler (p. 259). It is easily extracted from yeast by washing with water; the residual mixture of enzymes and activators has been termed *apo-zymase*. The function of

co-enzyme is to transfer phosphoric acid to the sugar substrates during the fermentation.

(2) *Pyruvic Acid and Acetaldehyde*.—The optimal pH for sugar fermentation by living yeast is about 5–6. If the mixture be kept on the alkaline side of neutrality there is a steady accumulation of pyruvate instead of alcohol. Similarly, if sodium hydrogen sulphite (bisulphite) be added to the mixture, there is an accumulation of acetaldehyde, which is bound by union with the sulphite. Since pyruvic acid is transformed into acetaldehyde by *pyruvic carboxylase*, and since acetaldehyde when added to the fermenting mixture is hydrogenated to alcohol, it is concluded that these two compounds are successive intermediates in sugar fermentation.

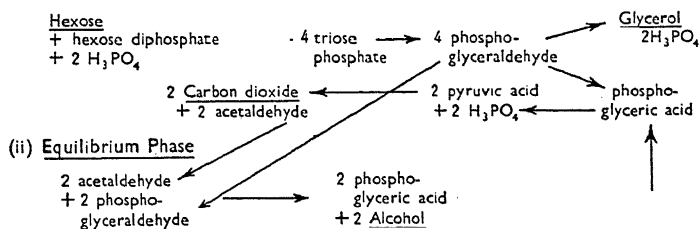
(3) *Sugar Phosphates*.—Before the sugar is attacked it is converted into an unstable form by the enzyme *hexokinase*, which is present in yeast but not in muscle. Phosphorylation accompanies this process, and the hexose phosphate is resolved into two molecules of triose phosphate.

(4) *Glycerol* is an invariable by-product of sugar fermentation. It appears in the early stages of the process, and reaches a maximum concentration which does not change appreciably during prolonged glycolysis. However, if fermentation be checked by the addition of sulphite, which does not allow it to proceed beyond the aldehyde stage, there is a steady increase in the production of glycerol. Under these conditions the end-products of sugar fermentation by yeast are glycerol, acetaldehyde and carbon dioxide, a consequence which proved of national, though not international, value in providing the central European countries with glycerol for explosive manufacture during the war period of 1914–1918.

The early formation of glycerol is explained by the dual nature of the fermentation process, which consists of: (i.) an initial phase before aldehyde has been formed, and (ii.) an equilibrium phase when aldehyde is interacting with the glycerol precursor, phosphoglyceraldehyde. These phases have been elucidated largely by the work of Embden, Meyerhof and Kieserling.

(i) Initial Phase

Sugar Fermentation Cycle



Meyerhof has shown that hexose phosphorylation requires Mg ions and co-enzyme, which reacts with the sugar to yield hexose diphosphate and adenylic acid in a manner comparable to the action of adenine triphosphate as a phosphate carrier in muscle glycolysis.

THE GLUCO-KINETIC HORMONES

The blood sugar level is maintained in accordance with the needs of the organism by the co-action of three hormones: adrenaline, insulin and the glycotropic factor of the anterior pituitary gland.

Adrenaline (adrenine), or epinephrine, influences carbohydrate traffic in three ways:—

- (a) Acceleration of hydrolysis of liver glycogen to glucose, an effect antagonised by insulin.
- (b) Acceleration of conversion of muscle glycogen to lactate.
- (c) Retardation of glucose acceptance by peripheral tissues.

Insulin.—Insulin, the chief autacoid of the pancreas, controls carbohydrate metabolism by rendering the blood sugar more mobile, perhaps by a direct activation of the sugar molecule or by a sensitising action on the tissues. Evidence suggests that insulin produces an unstable modification of glucose which is readily polymerised to glycogen or oxidised to simpler metabolites. This effect, however, cannot be demonstrated *in vitro*. There are two principal responses to insulin injection:—

- (a) Increased conversion of glucose to glycogen in the liver.
- (b) Increased acceptance and oxidation of glucose by all or most of the tissues.

The combination of both these effects results in a rapid fall in the blood sugar level, which may go below the minimal limit of 40–50 mg. per 100 ml., and induce *hypoglycæmia*, characterised by severe convulsions. These are rapidly relieved by glucose administration.

When insulin is injected into a normal animal the removal of glucose by the tissues is usually so rapid that the liver glycogen is broken down in an endeavour to maintain the glucose level in the blood.

Muscle glycogen is not decreased under these conditions, showing that the essential function of this polysaccharide differs in the different tissues. Only hepatic glycogen can support directly the blood sugar concentration.

Pituitary Factor.—In 1930, Houssay showed that removal of the pituitary gland antagonised the glycosuria and ketogenesis in depancreatized dogs; and Burn and Ling showed that injection of extracts of the anterior pituitary was able to evoke ketogenesis in normal animals. From this it was concluded that the gland

had a stimulating effect on fat metabolism which might lead to ketone formation when inadequately balanced by carbohydrate utilisation. That the effect is more complex is shown by the results of hypophysectomy in otherwise normal animals. In general, it appears to depress the secretion of sugar by the liver. Hypophysectomised animals readily develop hypoglycæmia, and are very sensitive to carbohydrate starvation and to insulin injection. Conversely, adrenaline injection has very little effect in raising the blood sugar level, even when the hepatic glycogen store is ample, which suggests that the pituitary factor is necessary for hepatic glycogenolysis in dogs.

The hypoglycæmia due to phloridzin injections or starvation can be alleviated in hypophysectomised dogs by feeding with carbohydrate or protein, but not by feeding with fat, which has led Soskin (1935) to conclude that the pituitary factor promotes the conversion of fatty acids into sugar.

The combined effects of loss of both pancreas and pituitary gland are demonstrated in the Houssay animals, which may live as long as nine months without special treatment although in a state precariously poised between fatal hypoglycæmia and diabetes. The glycosuria varies between 0.5 and 10 gm. *per diem*, as compared with the 50–70 gm. excreted daily on a similar diet by a dog from which the pancreas alone has been removed. Ketonuria is slight, and the glycogen levels in liver and muscle are normal. These remarkable phenomena indicate that the Houssay animal is able to utilise carbohydrate in the complete absence of pancreatic insulin.

Glycosuria.—The appearance of glucose in the urine, beyond the trace normally present, may be due to lowering of the renal threshold either congenitally (renal glycosuria) or experimentally (phloridzin glycosuria), or may be due to metabolic conditions which evoke hyperglycæmia. These include: (i.) alimentary glycosuria, which is rare in normal subjects; (ii.) insulin deficiency due to removal or disease of the pancreas (pancreatic diabetes); (iii.) excessive hepatic glycogenolysis, which may follow injection of adrenaline, pituitary extract, thyroxine, or may arise from the release of these hormones in asphyxia, general anaesthesia, emotional stress, toxic goitre, acromegaly, and experimental injury to the floor of the fourth ventricle (puncture diabetes).

The clinical condition, diabetes mellitus, is ascribed to pancreatic dysfunction leading to insulin deficiency.

Lactose Formation in the Animal Body.—Lactose, the characteristic disaccharide of milk, is a foreign sugar within the organism, and when injected into the circulation is excreted by the kidney, a

phenomenon seen in the overflow lactosuria that accompanies active lactation. This indicates that the sugar must be assembled locally in the mammary gland and secreted directly in the milk. The amount of glucose removed from the blood by the lactating gland depends on the arterial level of blood sugar, which is relatively constant for the species.

The precursors of lactose are glucose, removed directly from the blood, and lactic acid, some of which is manufactured in the lactating gland (Graham, 1937).

GENERAL REFERENCES

- CORI, C. F. (1931), "Mammalian carbohydrate metabolism." *Physiol. Rev.*, **11**, 143.
- CORI, C. F., and G. T. CORI (1935), "Carbohydrate Metabolism." *Textbook of Biochemistry*. Ed. Harrow and Sherwin, 536.
- DEUEL, H. J. (1936), "Intermediate metabolism of fructose and galactose." *Physiol. Rev.*, **16**, 173.
- DIXON, K. C. (1937), "The Pasteur effect and its mechanism." *Biol. Rev.*, **12**, 431.
- EGGLETON, P. (1929), "The position of phosphorus in the chemical mechanism of muscular contraction." *Physiol. Rev.*, **9**, 432.
- HILL, A. V. (1933), "Living Machinery." London.
- HOLMES, E. (1937), "The Metabolism of Living Tissue." Cambridge.
- HOPKINS, R. H., and B. KRAUSE (1937), "Biochemistry applied to Malting and Brewing." London.
- MACLEOD, J. J. R. (1926), "Carbohydrate metabolism and Insulin." London.
- MANN, F. C., and J. L. BOLLMAN (1939), "Physiology of the liver." *Ann. Rev. Physiol.*, **1**, 267.
- OCHOA, S. (1938), "Aspects of the biochemistry of muscle." *Chem. Ind.*, **57**, 720; **57**, 910.
- YOUNG, F. G. (1936), "Glycogen and the metabolism of carbohydrate." *Lancet*, 231, 297.

CHAPTER XVII

INTERMEDIATE METABOLISM: PROTEINS

digestion amino acids are liberated from the hydrolysed food proteins and absorbed into the portal blood. The effective surface of the small intestine is enormously increased by the presence of the villi, and represents 8–10 sq. metres in the adult human subject. Amino acid absorption is ascribed to simple diffusion, and owing to the relative slowness of protein digestion, proceeds at about 1 gm. per kg. of body weight per hour (Pfüger).

It is possible that some of the smaller peptides are also absorbed. The amino acid content of human plasma is expressed in terms of amino nitrogen, measurement of the individual amino acids being impracticable in small samples. In the resting subject, plasma amino nitrogen is about 3 mg. per 100 ml. Values between 5.2 and 7.2 mg. have been reported for entire blood. Ingestion of protein foods results in a rise in the amino acid values within two hours of the meal, and reaches a maximum about four hours after the beginning of the meal. Circulating amino acids may be stored temporarily in the tissues during the absorption peak, and the concentration of tissue amino acids may rise considerably above the blood value maximum of about 10 mg. amino nitrogen per 100 ml.

General History of the Amino Acids.—After absorption and circulation, amino acids may undergo :—

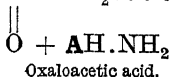
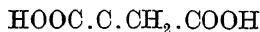
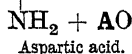
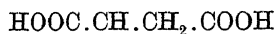
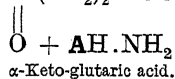
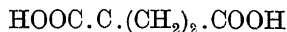
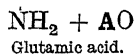
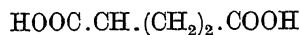
- (i.) Temporary storage in muscle, liver, kidney and other tissues.
- (ii.) Conversion into closely related derivatives.
- (iii.) Elaboration into tissue proteins.
- (iv.) Trans-amination, or exchange of amino groups.
- (v.) Deamination, or degradation by loss of the α -amino group.

As trans-amination and deamination are common events in the history of amino acids, they will be considered first.

Trans-amination.—Outside the organism, amino acids are relatively stable compounds; within the organism, the α -amino group becomes very reactive, and may be transferred to an amino acceptor, or liberated as ammonia by the agency of suitable catalysts. Amino transference was first observed by Braunstein and Kritzmann (1937), who named it “trans-amination.” It is a

reversible process, and is catalysed by the enzymes *glutamic* and *aspartic dehydrogenases* or *aminopherases*, which occur in muscle, liver, and many other tissues.

Trans-amination requires a specific amino donor, glutamic, or aspartic acid, or a specific amino acceptor, α -keto-glutaric acid, or oxaloacetic acid, according to the direction of the reaction.

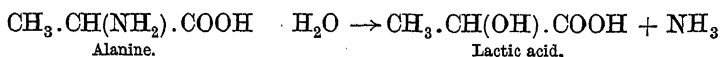


AO represents a specific amino acceptor, such as an α -keto acid. In the reverse reaction, $\text{AH} \cdot \text{NH}_2$ represents a suitable amino donor, which may be one of the natural amino acids. That is to say, the aminopherase system, working in one direction, can convert monocarboxy keto acids such as pyruvic, into amino acids by donation of $-\text{NH}_2$; and working in the reverse direction, aminopherases can deaminate amino acids to keto acids by removal of $-\text{NH}_2$. Such systems are of obvious importance in the natural synthesis of amino acids from carbohydrate residues and products.

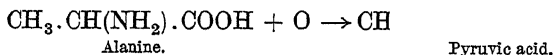
By feeding tyrosine containing the nitrogen isotope, ^{15}N , to normal rats, Schoenheimer has found that isotopic tyrosine can be incorporated into tissue proteins, and furthermore, that the isotope ^{15}N can be detected in other amino acids, showing that an exchange of amino nitrogen is continuously taking place (1939).

Deamination.—Amino acids can lose an α -amino group hydrolytically in two ways :—

(a) Direct hydrolysis to the corresponding hydroxy acid,



(b) Dehydrogenation (oxidation) to the corresponding keto acid,

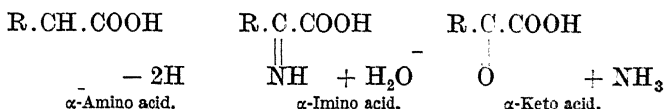


The frequent appearance of α -hydroxy acids in plant and animal tissues suggests that hydrolytic deamination is a biological process, and this is supported by the work of Weitzmann and Bergmann (1938), who have obtained the corresponding hydroxy

acids by the action of ultra-violet light on aqueous solutions of amino acids.

Oxidative deamination, however, is a well-established process. It can be brought about by the action of hydrogen peroxide, or by deaminating enzymes (amino acid dehydrogenases) present in certain tissues, and the resulting keto acids have been isolated and identified (Krebs, Bernheim).

The mechanism appears to involve : (i.) dehydrogenation of the amino group to an imino group ; and (ii.) hydrolysis of the imino compound to a keto acid and ammonia.



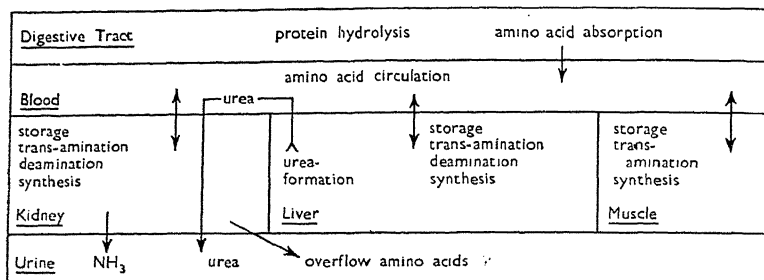
Deaminating enzymes occur in kidney cortex and, to a lesser extent, in liver. Vertebrate intestinal mucosa and cardiac muscle of pigeons and frogs have a slight and restricted capacity for deamination. Other vertebrate tissues (brain, retina, spleen, bone-marrow, pancreas, salivary gland and mammalian heart) appear to be incapable of deaminating amino acids. The ammonia released in deamination is either transformed locally in the liver into urea, or is transported to the liver by an ammonia carrier for conversion into urea. A small part of the renal ammonia escapes into the urine, and serves to regulate its H-ion concentration.

Braunstein believes that aminopherases provide the mechanism for amino acid synthesis and deamination in tissues that contain no general deaminases, and that aminopherases also may participate in the general deamination of the Krebs and Bernheim type that occurs in kidney and in liver.

The amino group of the mono-carboxylic amino acids is transferred to keto-glutaric acid, and the glutamic acid thus formed is readily deaminated in the tissue.

Tissue-protein Synthesis.—Since the tissue proteins are highly specific compounds, it is believed that the process of their elaboration from amino acids takes place within the cell on a catalytic surface (endo-enzyme) that in some way resembles the required protein pattern, and acts as a mould or pattern-die for the arrangement of the adsorbed amino acids. The protein assembled in a particular tissue may become part of the tissue structure or may be used for other purposes. Thus, the serum proteins are built up in the liver, the zymo-proteins are manufactured in the secreting glands, and caseinogen is manufactured in the mammary gland.

Summary of General Amino Acid Metabolism

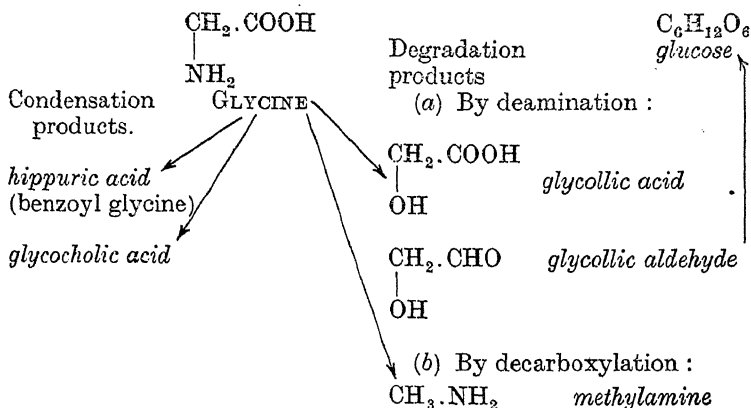


Ammonia Carriers.—Although ammonia appears as an end-product of general deamination, it is too toxic and reactive a metabolite to remain in the tissues in a free form, and is taken up by appropriate carriers, such as glutamic acid, aspartic acid, adenylic acid and adenosine, or may be removed by an aminophenylase system. These, or other carriers, transport ammonia to the liver for detoxication by conversion to urea.

Metabolism of the Individual Amino Acids.—Each amino acid has its own metabolic history in the organism, but few of these are known in more than outline. Inborn errors of metabolism, such as alcaptonuria, have led to the identification of intermediate products; and the increasing knowledge of the chemistry of the autacoids and alkaloids has shown that many of these are amino acid derivatives.

Glycine

Glycine is a non-essential amino acid, the animal being able to manufacture it when required. When administered to the diabetic

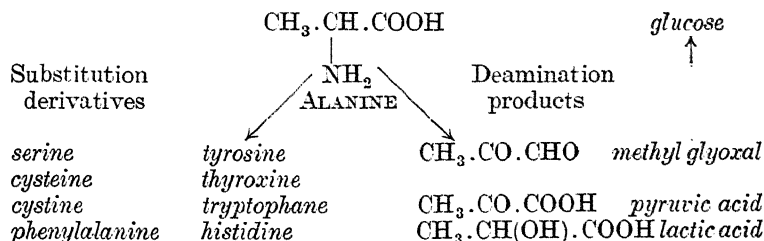


subject, glycine is converted into glucose, which provides a link between protein and carbohydrate metabolism. The chief derivatives of glycine in the higher animal are hippuric acid, the detoxication solute found in urine, and glycocholic acid, found in bile.

Both glycollic acid and aldehyde yield glucose when given to the diabetic animal, and are believed to be intermediate stages in the course of glycine metabolism. Methylamine is not produced in normal metabolism but may arise from alimentary putrefaction. Glycine has no effect in increasing the liver glycogen or raising the blood sugar level when fed to healthy animals.

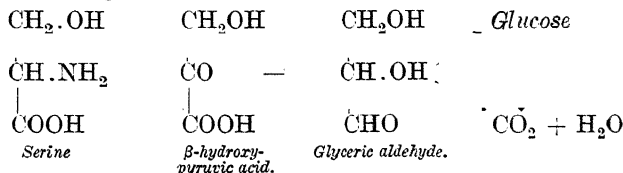
Alanine and Serine

Alanine is a precursor of glucose in the diabetic animal, and also is a non-essential acid. It is significant, however, in being related in structure, though not necessarily in biological derivation, to many more complex and important amino acids, and it readily transaminates with keto-glutaric acid.



glucose
↑

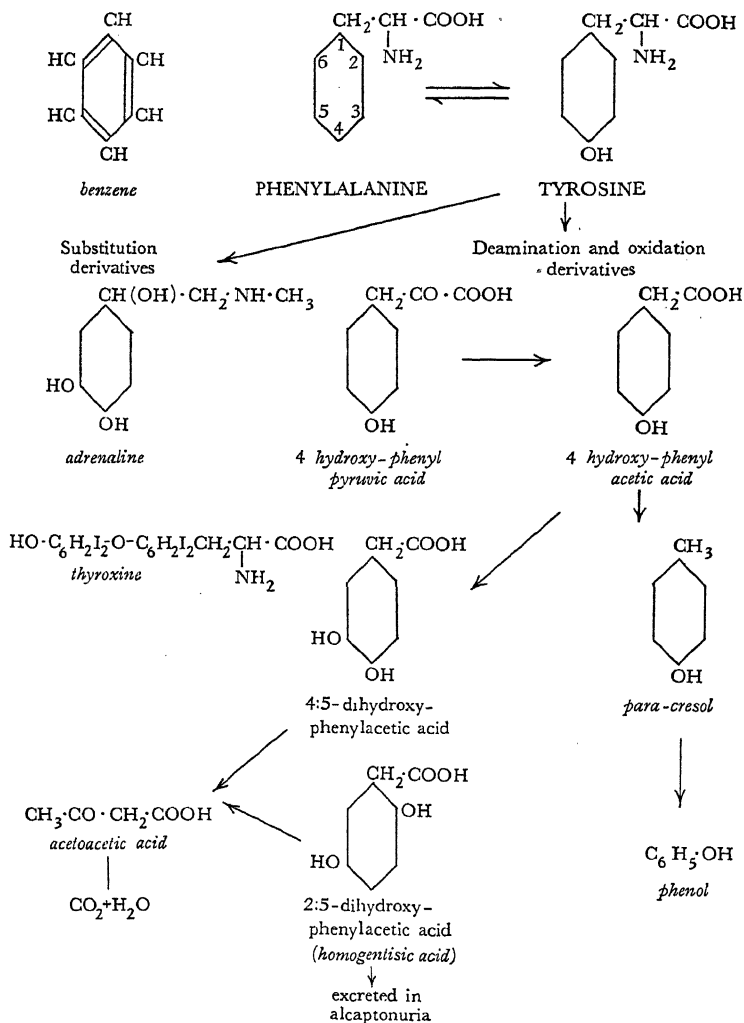
Serine is quantitatively converted into glucose in the diabetic animal, through the intermediate formation of glyceric aldehyde.



Phenylalanine and Tyrosine

These aromatic amino acids provide the chief source of the benzene ring, which, according to Abderhalden, cannot be synthesised by higher animals. Both amino acids yield acetoacetic acid when oxidised by liver tissue or when administered to diabetic subjects, and both yield *homogentisic* acid in alcaptonuric subjects. It is concluded that the amino acids are interchangeable in nutrition, and that phenylalanine is transformed into tyrosine previous to

metabolism. When phenylalanine is given to patients suffering from the inborn metabolic disease, *tyrosinosis*, it is excreted in the urine as tryosine (Medes, 1932). Phenylpyruvic acid, the deamination derivative of phenylalanine, does not yield acetoacetic acid in the diabetic animal, whereas *p*-hydroxy-phenylpyruvic acid



Tyrosine Metabolism

and homogentisic acid both yield acetoacetic acid, and thus are possible intermediates in tyrosine metabolism. From phenylalanine or tyrosine are derived the important hormones adrenaline and thyroxine, and the vaso-pressor tyramine, and, by bacterial degradation in the intestine, cresol and phenol, which appear as sulphates in the urine.

Melanin Pigments.—Tyrosine is oxidised by the enzyme tyrosinase to a red indole compound formed by closure of the side-chain. This red compound is reduced spontaneously to an indole base, *melanogen*, which subsequent oxidation polymerises to a dark-brown pigment, *melanin*. Melanin, or closely related melanoids, impart the characteristic colour to brown or black hair, the fur of animals, the choroid coat of the eye, the ink secretion of the cuttle fish *Sepia*, and the malignant melanotic sarcomata. The pigments are very stable, and are insoluble in water, acids, and organic solvents, but bleached by oxidising agents, including hydrogen peroxide. The changes involved in melanin formation have been elucidated by Raper (1928).

This series of reactions is of importance in establishing a relationship between tryptophane and tyrosine. The colourless melanogen is excreted in the urine of patients suffering from melanotic sarcoma, and may be recognised by its oxidation to melanin, which occurs spontaneously on exposure to the atmosphere, or after addition of any mild oxidising agents. A corresponding melanin is obtained from the plant amino acid, 3:4-dihydroxy-phenylalanine, by the specific enzyme, "*dopa*" oxidase, which has no action on tyrosine. The condition of albinism is ascribed to the lack of one or more of these oxidising enzymes.

Melanin formation in the deeper layers of the epidermis is responsible for the racial and climatic pigmentation of the skin, and may have some protective effect against excess of harmful solar radiation.

Tryptophane

Tryptophane is an essential amino acid, and provides the only known source of the indole nucleus in the diet.

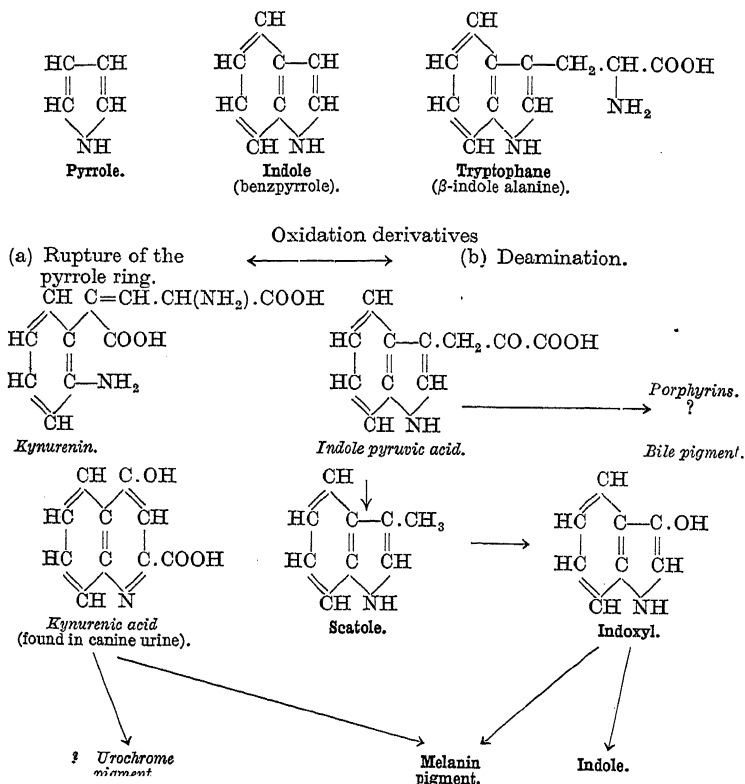
While no important biological reactant has yet been traced to tryptophane, it is probable that it supplies pyrrole nuclei for the synthesis of the porphyrins, including the essential cytochrome and hæmatin.

Tryptophane is the parent of the putrefaction products *scatole* and *indole*, and their oxidation derivative *indoxyl*.

Indole is present in the contents of the large intestine. It gives many colour reactions, notably :

(a) With Na nitroprusside in alkaline solution, a violet colour turning blue on acidification with acetic acid.

(b) With Ehrlich's aldehyde reagent in weak acid solution, a red colour, stable on dilution. Scatole only reacts in presence of excess of acid, and an oxidiser.

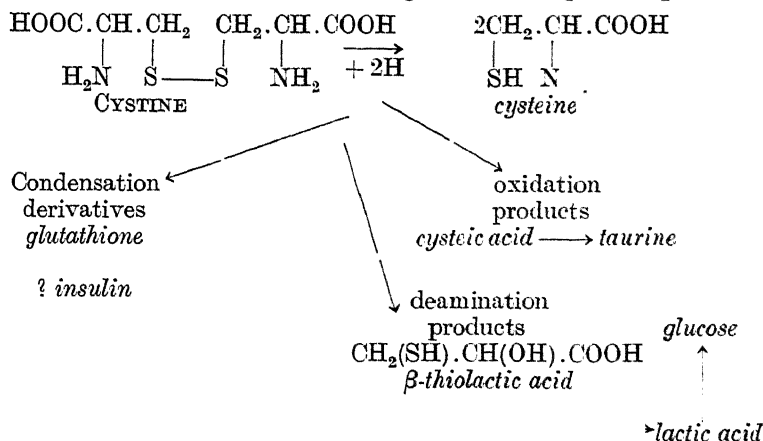


Sulphur-containing Amino Acids

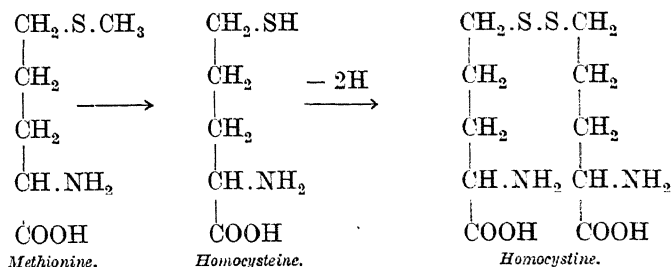
These include the natural amino acids, cystine and methionine, and their derivatives, cysteine, homocysteine, homocystine, and the tripeptide glutathione.

Cystine, the chief sulphur-containing amino acid, is the parent of many sulphur derivatives in the organism, and is an important constituent of the dietary. On reduction it is converted into two molecules of the amino acid cysteine, which contains sulphur as the highly reactive *thiol* group, $-\text{SH}$. Cysteine is completely con-

verted into glucose when administered in phloridzin diabetes; in this respect it resembles alanine and serine. Sulphur liberated from cysteine is the chief source of the organic and inorganic sulphates.



When administered to subjects of the metabolic disease *cystinuria*, cysteine, homocysteine and methionine are excreted largely as additional cysteine; whereas administered cystine, homocystine and glutathione are almost completely oxidised. From these observations it is concluded that cystine can be metabolised without previous reduction to cysteine, and that glutathione can be metabolised without previous hydrolysis, indicating that the metabolic history of an amino acid may depend on whether it is free or combined. Methionine, previous to its conversion into cysteine is demethylated to form homocysteine, which may undergo condensation to homocystine or degradation to simpler products.



Glutamic Acid and Proline

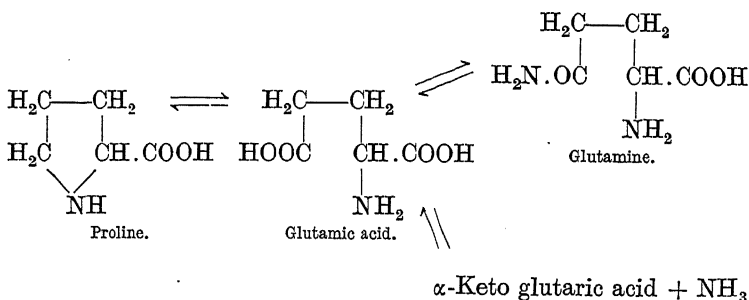
Neither glutamic acid nor hydroxy-glutamic acid has been found essential in nutrition although the former is a component of an

aminophorase system and also acts as a specific ammonia carrier in the metabolism of nervous tissue and in the formation of the urea in the liver.

In presence of glutamic acid, retina and brain cortex release ammonia in quantities up to 0.8 per cent. of the dry weight of the tissue, per hour, the ammonia being fixed as glutamine. A similar reaction has been observed in sheep, and rat kidney, though not in the kidney tissue of cat, dog, sheep or pigeon. Apart from this, glutamine formation appears to be restricted to the nervous system. The reaction is due to the specific enzyme, *glutaminase*, that under appropriate conditions aminates glutamic acid or deaminates glutamine.

Both glutamic acid and hydroxy-glutamic acid are glucogenic in the diabetic animal, three of the five carbon atoms being used for glucose formation, probably by way of α -keto glutaric acid.

Proline, a non-essential glucogenic amino acid, is oxidised to glutamic acid by kidney or liver tissue, and thus forms part of the glutamic cycle. Hydroxy-proline may undergo reduction to proline, but most of it appears to follow an individual metabolic path, as shown by the fact that, unlike proline, it can be converted into acetoacetic acid by liver tissue.



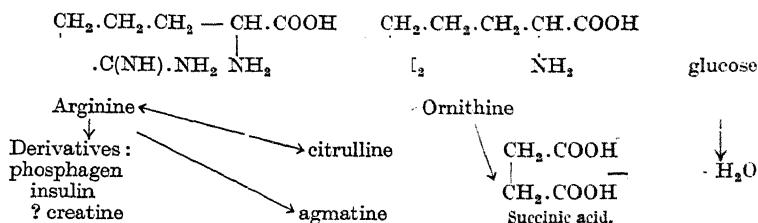
The conversion of glutamic acid to α -keto glutaric acid is catalysed by a highly specific enzyme, glutamic dehydrogenase, found chiefly in liver and kidney.

Arginine

Arginine, the amino acid derived from guanidine, is a probable constituent of all proteins, and can be synthesised by the animal body. Among mammals and many invertebrates, arginine takes part in the ornithine cycle whereby urea is assembled in the liver. Among invertebrates, arginine phosphate replaces creatine phosphate as the phosphagen of muscle, and arginine itself has been

regarded as a possible precursor of creatine in vertebrate metabolism. Arginine is hydrolysed by arginase to ornithine and urea.

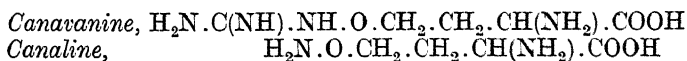
Both arginine and ornithine yield glucose in the diabetic animal, it is believed through the intermediate formation of succinic acid. Arginine is a constituent of insulin, and may contribute to the hormone the hypoglycæmic property possessed by other guanidine derivatives. On decarboxylation, arginine is converted into the corresponding amine, *agmatine*, a pressor base found among products of protein putrefaction.



Growth requirements of young animals may exceed their ability to manufacture arginine, and the acid is consequently an important constituent of their diets.

Canavanine and Canaline

These amino acids, originally obtained from the Jack bean, have the same relationship to each other as arginine has to ornithine, and like them can participate in the hepatic synthesis of urea. They have not been shown to be essential constituents of the animal dietary.



Histidine

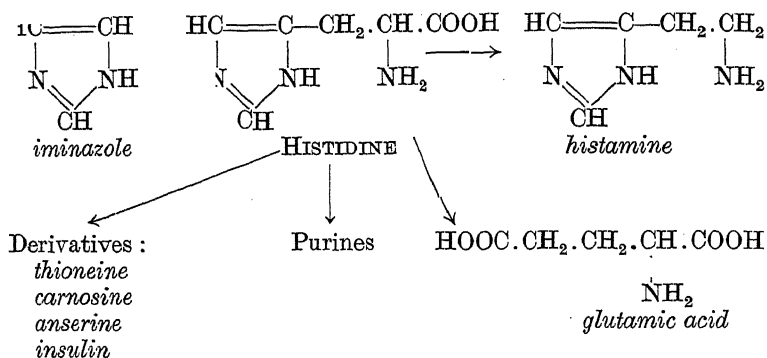
Histidine is an essential amino acid in nutrition, and provides the iminazole ring necessary for synthesis of purines and nucleoproteins. It forms neither glucose nor acetoacetic acid in the diabetic animal, though it is attacked by *histidase*, the enzyme present in vertebrate liver, which converts it into a compound that yields glutamate on alkaline hydrolysis.

Histidinuria is a characteristic accompaniment of pregnancy in the human subject. Histidine appears in the urine in the fifth week and ceases three days after birth. Histidase is absent from the livers of subjects dying during pregnancy, which has led Kapeller-Adler to conclude that the histidinuria is due to protective inactiva-

tion of the enzyme in order to conserve the amino acid for foetal requirements.

The principal derivatives of histidine in the animal body are (i.) *thioneine*, the betaine base of thiolhistidine (p. 362), which occurs in blood corpuscles; (ii.) *carnosine*; and (iii.) *anserine*, dipeptides found in muscle (p. 374). On decarboxylation, histidine forms the corresponding amine, histamine, the capillary dilator present in tissue extracts, and the chief factor in vascular shock.

Iminazole, the cyclic component of histidine, is not utilised appreciably in animal metabolism, and can be recovered almost quantitatively from the urine after injection (Leiter, 1925).



Nutritional Significance of the Amino Acids.—By means of feeding experiments on normal and diabetic animals it is possible to determine which amino acids are essential for life, and also which are capable of being converted into glucose or into acetoacetic acid. Experiments on the degradation of amino acids by surviving tissues afford information as to the course of their metabolism, and this is supplemented by a study of the inborn metabolic diseases in which the subject is unable to metabolise one or more of the natural amino acids.

Essential Amino Acids.	Non-essential Amino Acids.	Amino Acids of Unknown Nutritional Importance.
Arginine (?) . . .	Glycine.	Alanine.
Threonine . . .	Cystine.	Serine.
Lysine . . .	Citrulline.	Leucine.
Methionine . . .	Proline.	Isolucine.
Tyrosine	Hydroxyproline.	Norlucine.
(or Phenylalanine) .	Aspartic acid.	Canavanine.
Tryptophane . . .	Glutamic acid.	
Histidine. . . .	Hydroxyglutamic acid.	
Valine.		

Inborn Errors of Metabolism.—These conditions are due to faulty enzyme inheritance, and persist throughout life. They are characterised either by the inability to utilise normal nutrients or to manufacture normal products.

Albinism.—An inability to manufacture the melanin pigment found in hair and in the choroid of the eye. Albinism is frequent in animals such as rats, mice and rabbits.

Alcaptonuria.—An inability to metabolise all the phenylalanine and tyrosine of the diet, the surplus being excreted as homogentisic acid in the urine.

Tyrosinosis.—An inability to metabolise all the phenylalanine and tyrosine of the diet, the surplus being excreted as tyrosine or its deamination product, hydroxyphenyl pyruvic acid.

Cystinuria.—An inability to metabolise a portion of the cystine and methionine of the diet, although additional free cystine may be oxidised completely after ingestion.

Pentosuria.—The manufacture and excretion of a non-utilisable pentose, generally l-xyloketose, owing to a perversion of sugar metabolism.

Porphyria.—The manufacture and excretion of an abnormal pigment, uroporphyrin. Both alcaptonuria and porphyria have long been recognised, as shown by mediæval references to black and to red urine.

GENERAL REFERENCES

- Σ, A. E. (1939), "Transamination." *Nature*, **141**, 748.
 CUTHBERTSON, D. P. (1938), "Influence of carbohydrates on protein metabolism." *Chem. Ind.*, **57**, 815.
 KREBS, H. A. (1934), "Urea formation in the animal body." *Ergebnisse der Enzymforschung*, **3**, 247.
 KREBS, H. A. (1936), "Metabolism of amino acids." *Ann. Rev. Biochem.*, **5**, 247.
 MITCHELL, H. H., and T. S. HAMILTON (1929), "The Biochemistry of the Amino Acids." London.
 PETERSON, J. M. (1934), "The metabolism of eviscerate preparations." *Physiol. Rev.*, **14**, 586.
 RAPER, H. S. (1928), "Tyrosine and tyrosinase." *Physiol. Rev.*, **8**, 245.
 ROSE, W. C. (1938), "Nutritive significance of the Amino Acids." *Physiol. Rev.*, **18**, 109.

CHAPTER XVIII

INTERMEDIATE METABOLISM : LIPIDES

DURING digestion, the aliphatic acids of the simple and complex lipides are liberated, and by the time the intestinal contents have reached the cæcum very little fat remains unabsorbed.

The emulsified aliphatic acids form water-soluble dispersions with the bile salts, and pass readily across the frontier of the intestinal mucosa. The glycerol is phosphorylated during its absorption in a manner comparable to the hexoses, and in the cells of the mucosa it recombines with the aliphatic acids to form fat. For this reason, a hormone of the adrenal cortex, in some way associated with phosphorylation, is concerned in fat absorption, and phosphatase-inhibitors, such as phloridzin, retard the absorption process.

After re-synthesis, about 60 per cent. of the absorbed fat enters the lymphatics, the remainder travels in the portal blood, partly in the form of a very fine emulsion. The diversion of the lipides into the lymphatic stream enables fats to enter the general circulation before being immobilised by the liver.

The total lipide content of the blood is very variable and is made up of many components, some of which are not clearly defined. Two per cent. is maximal for normal subjects, but diabetic blood may contain as much as 20 per cent.

Lipide Distribution in Blood (Expressed in mg. per 100 ml.)

	Whole Blood.	Plasma or Serum.
Total lipides . . .	300-2,000	450-1,260
Fatty acids . . .	290- 410	190- 640
Phosphatides . . .	250- 450	175- 330
Cholesterol . . .	100- 230	100- 230

Total lipide is taken as represented by the total ether-soluble fraction of the material. Phosphatide is represented by the value for lipide phosphorus multiplied by a factor, 25.

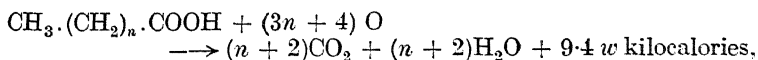
Kirk (1938) reports average values for human plasma to be : total fat, 559 ; total phosphatide, 145 ; lecithin, 19 ; cephalin, 68 ; expressed in mg. per 100 ml.

Absorbed fat travels in the blood in particles of about $1\text{ }\mu$ in diameter, termed "chylomicrons." Like the fat in milk, they are stabilised by being surrounded by a protective protein layer. They may be enumerated microscopically, and afford an index of the rate of fat absorption and utilisation. The fat content of the systemic blood rises in one to three hours after a meal rich in fat, reaches a peak in six or seven hours, and gradually subsides.

Structural Lipide and Storage Lipide.—In the organism the lipides are immobilised in two ways : (i.) as a structural constituent of cells and tissues, and (ii.) as the storage fat of adipose tissues. The former is determined by the growth requirements of the organism, the latter depends partly on the fat content of the diet and partly on the excess of food consumed above the nutritional requirements of the animal. The constitution of the acids in storage fat depends on the food. Oleic, palmitic, and stearic acid can be formed from excess carbohydrate, or from protein, probably through a carbohydrate intermediate stage. Unwanted lipides and sterols are excreted into the intestine, partly by the bile and partly by the mucosa of the large intestine. These make up 7.3–28 per cent. of the total faecal solids.

The Combustion of Fatty Acids.—Fats and their constituents provide an important source of energy to the organism. Unlike proteins, they can be stored in large amounts, and, unlike carbohydrates, their metabolism does not appear to be associated with any particular physiological process, such as muscular contraction.

The type equation for the complete combustion of a gram molecule of a fatty acid is :—



where w is the molecular weight of the fatty acid in grams. Each gram of higher fatty acid completely oxidised liberates 9.4 kilocalories of heat, or, each gram of fat liberates about 9.3 kilocalories, since it is a glyceryl ester of the acid.

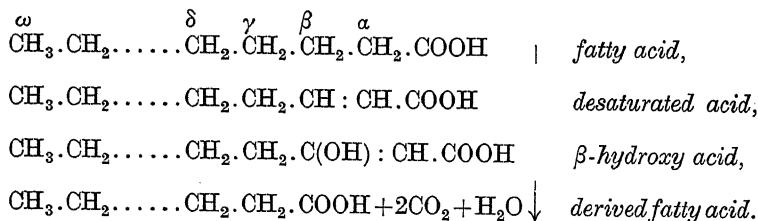
Desaturation of Fatty Acids.—The liver is very rich in unsaturated lipides, and it is believed that fat metabolism is located chiefly in this organ. A stage in fat degradation is the formation of a glycerophosphate ester or phospholipide (p. 168), either in the intestinal mucosa during fat absorption or in the liver after absorption. These esters contain two fatty acid radicles, one of which is unsaturated in the majority of the natural phospholipides, and it is

probable that desaturation of the acid radicle is a primary stage in fat degradation.

Using fats containing deuterium as an indicator, Raper and Cavanagh (1939) find a rise in the deuterium content of hepatic glycerides and phospholipides after fat absorption, and conclude that they are actively associated with early fat metabolism.

Oxidation of Fatty Acids.—The molecule of an unsaturated fatty acid such as oleic, is most likely to undergo oxidative attack at the point of unsaturation; the saturated acids, stearic and palmitic, are degraded by terminal oxidation. Fat oxidation occurs chiefly, if not entirely, in the liver, and under normal conditions the process is complete, and ends in CO_2 and H_2O . However, in diabetes and other conditions of carbohydrate inadequacy, fat oxidation in the liver is unable to proceed beyond acetoacetic acid, which suggests that this compound is an intermediate in fat metabolism. The natural fatty acids almost without exception contain an even total number of carbon atoms, and to explain the process of acetoacetic formation, Knoop proposed, in 1904, his theory of β -oxidation of the fatty acids, according to which, the point of oxidative attack is the carbon atom in the β -position, or next but one to the terminal carboxyl group. By this means the fatty acids are degraded two carbon atoms at a time.

β -Oxidation.



By a series of successive β -oxidations, stearic acid, $\text{C}_{17}\text{H}_{35} \cdot \text{COOH}$, is converted into palmitic acid, $\text{C}_{15}\text{H}_{31} \cdot \text{COOH}$; myristic acid, $\text{C}_{13}\text{H}_{27} \cdot \text{COOH}$; lauric acid, $\text{C}_{11}\text{H}_{23} \cdot \text{COOH}$; and, eventually, butyric acid, $\text{C}_3\text{H}_7 \cdot \text{COOH}$; acetic acid, $\text{CH}_3 \cdot \text{COOH}$; and carbon dioxide and water.

The phenomenon of β -oxidation affords an explanation why the fatty acids found in natural fats invariably contain an even number of carbon atoms. Fat synthesis presumably follows the same line as fat degradation, and proceeds by advances of two carbon atoms at a time.

(1) By feeding dogs with benzene compounds containing fatty acid side-chains, Knoop was able to recover the corresponding β -oxidation derivatives from the urine.

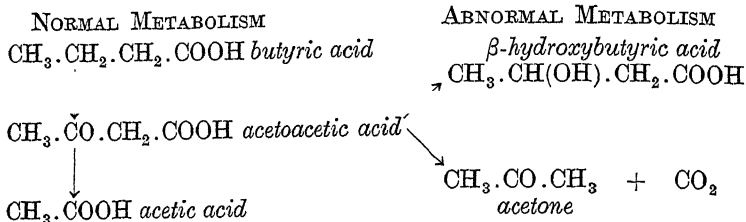
Thus, phenyl propionic acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH$, and phenyl valeric acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$, both yield benzoic acid, $C_6H_5 \cdot COOH$ (which is eliminated in the detoxified form as hippuric acid); while phenyl acetic acid, $C_6H_5 \cdot CH_2 \cdot COOH$, phenyl butyric acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$, and phenyl caproic acid, $C_6H_5 \cdot (CH_2)_5 \cdot COOH$, are all excreted as phenyl acetic acid (eliminated in the detoxified form as phenaceturic acid). The experiment is an interesting example of the use of the stable benzene ring as a means of tracing an organic residue during its metabolic experiences.

(2) Using hydrogen peroxide and a copper catalyst, aliphatic acids can be converted into β -oxidation products, showing that this part of the molecule is sensitive to attack.

(3) By perfusion of the surviving mammalian liver with salts of fatty acids, β -oxidation products can be obtained.

(4) The appearance of β -hydroxybutyric acid, acetoacetic acid, and acetone in the blood and urine of diabetics is explained most readily by the β -oxidation theory of fat metabolism.

Ketogenesis and Ketosis.—The penultimate stages of fatty acid oxidation are linked in some way with sugar metabolism and require the presence of active blood sugar; that is to say, glucose and insulin are necessary for normal fat metabolism. In conditions of glucose shortage, as in severe carbohydrate starvation, and in conditions of insulin deficiency, as in diabetes mellitus, the oxidation of the fatty acids stops short with the production of *acetoacetic acid*, $CH_3CO \cdot CH_2 \cdot COOH$, the β -oxidation derivative of butyric acid. Oxidation cannot proceed further, and the organism endeavours to get rid of the accumulating acetoacetic acid by two other biochemical processes. In part it is reduced to *β -hydroxybutyric acid*, and in part it is decarboxylated to *acetone*. All three metabolites enter the blood stream and appear in the urine, the conditions being termed *ketonæmia* and *ketonuria*, because two of the compounds, namely, acetoacetic acid and acetone, carry a ketone group. Normally, the ketone value of blood is less than 1 mg. per 100 ml., but in diabetic ketogenesis it may reach 300 mg. or more, corresponding to a urinary excretion of as much as 75 gm. *per diem*. These ketones are toxic, and their accumulation in the blood and tissues gives rise to the condition of *ketosis*, or ketone poisoning accompanied by acidoses, which leads to coma and death. Administration of glucose and insulin is the specific treatment of ketosis, since they provide the missing factor in fatty acid oxidation, and enable the organism to metabolise acetoacetic acid. The detection and identification of these ketones is described in connection with urine analysis, in Chapter XXIII.



Terminal Stages in Fatty Acid Oxidation

Multiple Alternate Oxidation.—Fatty acids with a carboxyl group at each end of the hydrocarbon chain have been found in the urine after fat ingestion, which shows that β -oxidation is not the only path traversed in fat metabolism, and that terminal or ω -oxidation also can occur. Furthermore, Jowett and Quastel (1935) have found that although liver tissue can form acetoacetic acid from a variety of acids containing an even number of carbon atoms, the rate of formation is less with butyric acid than with higher acids in the same series, which indicates that butyric acid is not an essential intermediate. They have proposed a theory of multiple alternate oxidation, according to which the fatty acid is oxidised at different points on alternate carbon atoms before the linkage is broken.

Using a fatty acid containing deuterium as indicator, Schoenheimer and Rittenberg (1936) have demonstrated that desaturation can take place in mice, but that this desaturation is restricted to particular molecular points is implied by the discovery of the Burrs and Evans (1932) that either linoleic or linolenic acid must be supplied in the diet of rodents in order to obtain normal growth and reproduction. This unsaturated component they term vitamin F (p. 251). If the animal were able to desaturate a fatty acid at any linkage, it is hard to understand how a nutritional disorder due to unsaturated acid deficiency can arise.

Stearic Acid: $\text{CH}_3 \cdot (\text{CH}_2)_{16} \cdot \text{COOH}$. Non-essential.

Oleic Acid: $\text{CH}_3 \cdot (\text{CH}_2)_7 \cdot \text{CH}=\text{CH} \cdot (\text{CH}_2)_7 \cdot \text{COOH}$. Non-essential.

Linoleic Acid: $\text{CH}_3 \cdot (\text{CH}_2)_4 \cdot \text{CH}=\text{CH} \cdot \text{CH}_2 \cdot \text{CH}=\text{CH}(\text{CH}_2)_7 \cdot \text{COOH}$.

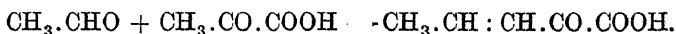
Linolenic Acid: $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}=\text{CH} \cdot \text{CH}_2 \cdot \text{CH}=\text{CH} \cdot \text{CH}_2 \cdot \text{CH}=\text{CH} \cdot (\text{CH}_2)_7 \cdot \text{COOH}$.

} Essential.

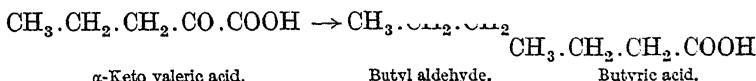
Interconvertibility of Fat in the Organism.—That tissue fat can arise from dietary carbohydrate was shown experimentally by

Lawes and Gilbert, in 1852, who found that pigs fed exclusively on barley acquired more body fat than could possibly have come from the fat or protein in the barley. A similar transformation is demonstrated, often unintentionally, by the human subject, and a restriction in the carbohydrate intake is a routine procedure in the treatment of obesity and over-weight conditions. Fat can also arise from protein, as shown by the work of Lusk and his colleagues on the effect of feeding excess of lean meat to dogs whose glycogen and lipide stores had been depleted by previous starvation.

Two factors are required in fat production : (i.) glycerol, and (ii.) the precursor of the fatty acids. Glycerol can arise during carbohydrate metabolism either directly from glucose or indirectly from the glucogenic amino acids. The precursor of the fatty acids is probably pyruvic acid, which is an intermediate in glucose degradation and also in alanine deamination. By decarboxylation of pyruvic acid, *acetaldehyde* is formed, and can undergo union with itself, by aldol condensation, or with pyruvic acid, to form an unsaturated 5-carbon aliphatic acid.

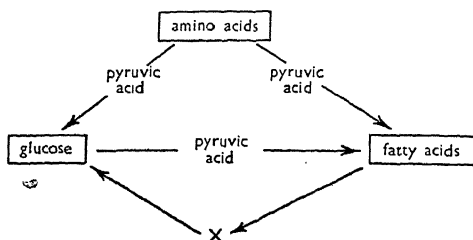


This may undergo reduction to the corresponding acid, α -keto valeric acid, or may be decarboxylated to an aldehyde, which then reacts with another molecule of pyruvic acid to form a 7-carbon acid. By a continuation of this process, fatty acids of increasing length can be assembled. Intermediate reactants such as these have not been found as yet in animal tissues, but the fact that milk fat contains a variety of lower members of the aliphatic series suggests that fat construction in the organism is by such stages. The preferential occurrence in nature of fatty acids containing an even total number of carbon atoms is explained by the decarboxylation and subsequent terminal oxidation of the antecedent keto acid.



The possible conversion of fat to carbohydrate within the organism has been a subject of much controversy. While the glycerol component of the fat molecule can be transformed readily into liver glycogen, a carbohydrate precursor has not yet been identified among the products of fatty acid metabolism. The existence of a pituitary factor in carbohydrate metabolism has been established, and one of its effects is ascribed to promotion of glycogenesis from fatty acids.

Inter-relationship of Protein, Carbohydrate and Fat



The Origin of Milk Fat.—Fat occurs in milk to the extent of 2–4 per cent. depending on the species of the animal and the dietary conditions. It differs from body fat in containing several of the lower fatty acids, about 7 per cent. of which are volatile in steam, and presumably have a special function in nutrition.

Expressed as percentage of total fatty acids, the composition of English dairy butter is: butyric acid, 4·4; caproic acid, 1·4; caprylic acid, 1·0; capric acid, 1·1; lauric acid, 3·5; myristic acid, 20·1; palmitic acid, 15·2; stearic acid, 1·1; oleic acid, 45·5 (Crowther and Hynd).

According to Graham, Jones and Kay (1936), milk fat is derived chiefly from the non-phospholipide fatty acids of the blood. Phospholipides are precursors neither of milk fats nor of milk phosphoproteins, which obtain their phosphate from the inorganic solutes of the plasma.

COMPLEX LIPIDES AND LIPOIDS

Phospholipides.—Lecithin and other phospholipides of the diet are important nutritional sources of choline and of phosphoric acid, and are resolved into their components by the esterases of the small intestine previous to absorption. Within the intestinal mucosa, a re-synthesis occurs, or a new phospholipide is assembled containing units derived from the saponified fats of the diet, and as such participates in the lipide transport in the lymph and the portal blood. By use of a phosphate containing a radioactive isotope of phosphorus as indicator, Artom and his colleagues (1937) have shown that phospholipides of the lecithin and cephalin type are synthesised in large quantities during fat absorption, and accumulate in the intestinal mucosa, the liver, and to a lesser extent, the kidney, but not in the spleen, heart or skeletal muscles.

That phospholipide is concerned in the hepatic metabolism of fat is suggested by the discovery that choline, the onium base present in lecithin, inhibits the deposition of excess fat in the liver, and is essential for this function in nutrition (p. 264). Choline

inhibits the formation of acetoacetic acid from fatty acids by liver tissue, and presumably diverts metabolism into channels other than storage or ketogenesis.

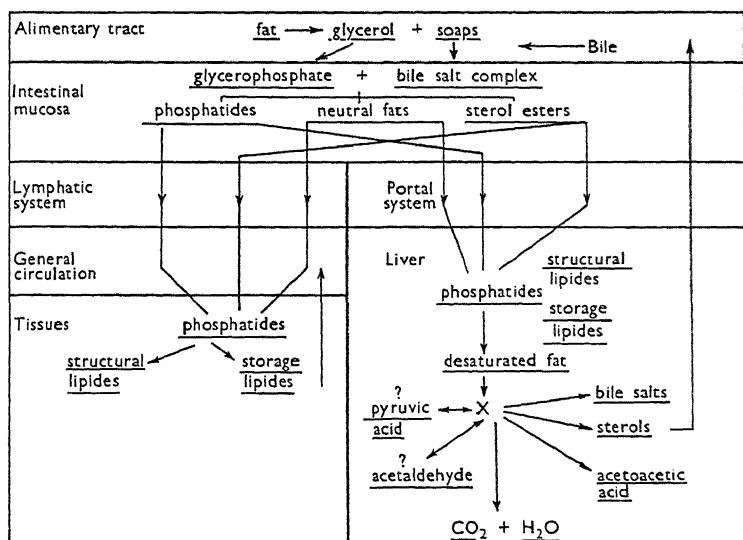
Sterols.—Cholesterol is readily and preferentially absorbed, and circulates as an ester; phytosterols are not absorbed, probably because the necessary esterifying system is absent from the intestinal mucosa. The D vitamins and the provitamin ergosterol are rapidly absorbed, but the potency of these compounds is so great that it is not easy to show how far their absorption is specific and quantitative.

Carotinoids.—The carotenes α , β and γ , are preferentially absorbed by ruminants, but the human intestine does not discriminate between them and the useless pigment xanthophyll, and both types of carotinoid circulate in human plasma.

The steroid hormones, androgens and oestrogens also are absorbed slowly, and Demole (1932) claims that their effect is diminished and delayed when they are given by the mouth.

Hydrocarbons.—Channon has shown that unsaponifiable hydrocarbons, phytol, higher alcohols, and liquid paraffin can be absorbed in small quantities from the intestine, depending on their solubility in bile acid mixtures. This has a practical significance on account of the widespread use of medicinal paraffins in the prolonged treatment of chronic intestinal conditions.

Summary of Fat Metabolism



GENERAL REFERENCES

- BLOOR, W. R. (1935), "Lipide Metabolism." *Textbook of Biochemistry*.
Ed. Harrow and Sherwin, 578.
- NISSEN, N. I. (1933), "Alimentary Lipæmia in Man." Copenhagen.
- SINCLAIR, R. G. (1934), "Physiology of the phospholipides." *Physiol. Rev.*, **14**, 351.
- SINCLAIR, R. G. (1937), "Fat metabolism." *Ann. Rev. Biochem.*, **6**, 243.
- VERKADE, P. E. (1938), "Role of dicarboxylic acids in metabolism." *Chem. Ind.*, **57**, 704.

CHAPTER XIX

TISSUE RESPIRATION

'Life is a pure flame, and we live by an invisible fire within us.'
THOMAS BROWNE.

: the physical standpoint, life is a peculiar and orderly way in which energy is transformed. The source of this energy is the food material of the cells, and it is released by two different ways : *degradation* and *oxidation*. By degradation is meant the fragmentation of the food molecule, usually under anaerobic conditions. By oxidation is meant the combustion of the molecule with the ultimate liberation of carbon dioxide and water. Degradation is represented by various natural fermentations, and the conversion of glycogen into lactic acid in contracting muscle. It is a primitive and uneconomical process, and is only able to provide energy for the simplest types of life.

"The development of more complicated and hence more pretentious forms of life became possible only after Nature discovered oxidation by molecular oxygen. This course of events is still reflected in our cells, in which we find oxidation and fermentation intimately mixed and woven into one energy-producing system."
(Szent-Györgyi, 1937.)

The term *respiration* is applied to the process whereby oxygen is utilised for the combustion of food molecules. General respiration is the sum of the activities of the entire organism as shown by the alteration in the oxygen and carbon dioxide content of the external environment ; tissue or cellular respiration is the utilisation of oxygen for the combustion of cell metabolites.

The general respiration of all higher plants and animals is aerobic ; lower organisms can live for varying but limited periods by incurring an oxygen debt due to the accumulation of lactate or other partially oxidised metabolites.

It was shown by Pflüger, in 1875, that frogs deprived of oxygen can survive for about seventeen hours, during the first five of which the rate of carbon dioxide elimination is normal, but subsequently decreases. These observations have been extended to other organisms, such as insects and parasitic worms, some of

which can oscillate readily between aerobic and anaerobic activity according to the amount of oxygen available. The obligatory anaerobes represent a type of monocellular organism which is unable to live in presence of free oxygen, a condition which O'Meara (1936) ascribes to the toxic effect of traces of copper in the culture media. Completely deprived of oxygen, man can only survive for about three minutes, unless specially trained to economise and endure.

Methods of Investigation.—Early work on tissue metabolism was carried out by means of extracts or suspensions of comminuted material. This has been replaced by the *tissue-slice* technique developed by Warburg and others, in which thin sections of fresh tissue are suspended in isotonic saline solutions containing various substrates. Under these conditions the structure of the tissue is preserved, and the cells remain active for several hours.

Aerobic respiration is measured directly by determining the oxygen uptake when the tissue section is placed in one cup of a Barcroft differential manometer. Anaerobic respiration is determined by incubating the material with methylene blue in a Thunberg tube containing nitrogen. This method is specially suitable for the study of dehydrogenase systems, the activity of which is found by measuring the rate of bleaching of the dye.

The Tissue Respiration Quotient.—Tissue respiration is expressed in terms of a special quotient, Q , which represents the quantity of substance produced or consumed per mg. dry weight of tissue per hour. Q , when possible, is expressed in cubic millimetres of gas (O_2 , CO_2 , NH_3) at n.t.p., and when negative indicates consumption or absorption by the tissue. Q is also qualified by the addition of suffixes, the lower of which specifies the substance transformed, and the upper denotes whether the conditions are aerobic (O_2) or anaerobic (N_2).

Thus, $Q_{O_2} = -7$ indicates that the tissue is consuming $7 \mu l$ (microlitres) or cu. mm. of oxygen per mg. dry weight of material per hour.

Similarly, $Q_{CO_2}^N = +20$ indicates that the tissue is liberating $20 \mu l$ of carbon dioxide per mg. dry weight per hour, in absence of oxygen.

A microlitre, μl , is the millionth part of a litre, or the thousandth part of a millilitre (ml.).

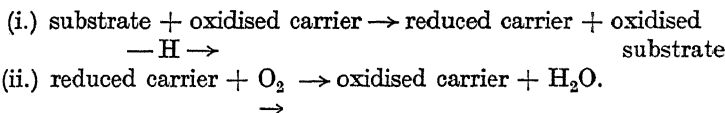
Representative Q_{O_2} values for rat tissues at body temperature are: liver, -7 ; kidney cortex, -25 ; brain cortex, -15 ; skeletal muscle, resting, -6 ; skeletal muscle, active, -40 . Holmes, from whose work these data are taken, observes that the reason for the high oxygen consumption on the part of some tissues

is obviously due to the fact that they have to do physical work. "The one exception is the grey matter of the central nervous system which, while it does no work which can be measured in terms of osmotic or mechanical energy, consumes a very considerable amount of oxygen." The human kidneys in concentrating 1.5 litres of urine in the twenty-four hours perform about 3,225 kilogram-metres of work, and have an oxygen consumption representing nearly 9 per cent. of that consumed by the entire body when at rest.

THE RESPIRATORY PROCESS

Glucose, lactate, succinate, aliphatic and amino acids, and other substances oxidised by animal tissues are very stable in solution, and are not obviously affected by atmospheric oxygen at ordinary temperatures. In the tissues, however, they are rapidly and effectively oxidised at body temperature, which for cold-blooded animals may be as low as 10° C. These combustions are brought about by a series of chain reactions, often of surprising complexity, which include (i.) respiratory catalysts, and (ii.) respiratory carriers.

Respiratory catalysts, represented by the *dehydrogenases*, combine with the food substrates, and unstabilise hydrogen atoms, which are then transferred to a series of appropriate hydrogen carriers, only the last of which interacts with free oxygen :—



Thus, the respiratory process involves two distinct systems :—

(a) The *dehydrogenase* system (Wieland-Thunberg), which activates hydrogen in the substrate.

(b) The *carrier-oxidase* system (Warburg-Keilin), which activates molecular oxygen so that it can oxidise the activated and transferred hydrogen.

The interdependence of the two systems has been elucidated by Green and by Szent-Györgyi, who has also discovered the existence of an intermediate group of hydrogen carriers represented by the C₄ dicarboxylic acids.

Oxidation may be regarded as (i.) the removal of hydrogen, as in the conversion of alcohol to aldehyde,

or (ii.) the addition of oxygen, as in the conversion of aldehyde to acid, $\text{R} \cdot \text{CHO} + \text{O} \rightarrow \text{R} \cdot \text{COOH}$. In the majority of tissue oxida-

tions, the first change undergone by the substrate is removal of hydrogen. The dehydrogenated residue, being more reactive than the original substrate, is subsequently oxidised or hydrolysed by the action of other catalysts.

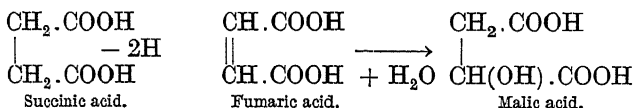
The Respiratory Catalysts

The thermo-labile respiration catalysts include :—

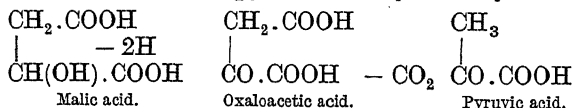
- A. *Dehydrogenases*, which activate the substrate so that hydrogen is transferred to the appropriate carrier.
- B. *Oxidases*, which activate the substrate so that it can be oxidised directly by molecular oxygen.
- C. *Peroxi-dases*, which transfer oxygen from peroxides to oxidisable substrates without the liberation of free oxygen.
- D. *Catalase*, which decomposes hydrogen peroxide into water and oxygen.

A. **Dehydrogenases**, dehydrases, oxido-reductases or hydrogen-transportases are widely distributed in vertebrate, invertebrate and plant tissues. Most of them are highly specific enzymes, and operate in association with respiratory carriers, which are much less specific. As a class, they are inhibited by narcotics, but not by cyanide. Important examples are :

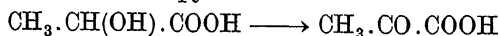
(1) **Succinic dehydrogenase**, present in most animal tissues, converts succinic acid into fumaric acid, which is subsequently hydrated to malic acid by *fumarase*, an enzyme accompanying succinic dehydrase, and one of the most powerful catalysts in the organism.



(2) **Malic dehydrogenase** oxidises malic to oxaloacetic acid, which is subsequently changed to pyruvic acid by carboxylase.

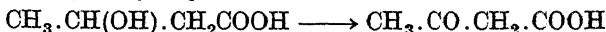


(3) **Lactic dehydrogenase**, accompanied by its co-enzyme, is present in muscle, brain and other tissues, and in yeast. It dehydrogenates lactic into pyruvic acid.

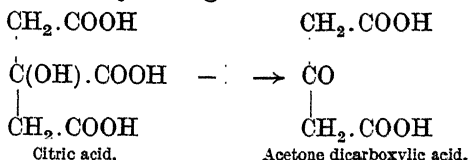


Narcotic drugs, especially barbiturates, inhibit lactic dehydrogenase in brain cortex, apparently by competing with it for the substrate (Quastel and Wheatley, 1933).

(4) **β -Hydroxybutyric dehydrogenase**, from liver and muscle, converts β -hydroxybutyric into acetoacetic acid.

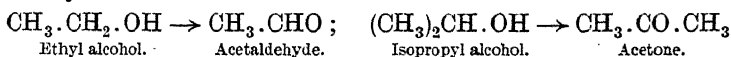


(5) **Citric dehydrogenase**, from liver, muscle and vegetable extracts, converts citric acid into acetone dicarboxylic acid, which by a second decarboxylation gives rise to acetoacetic acid.

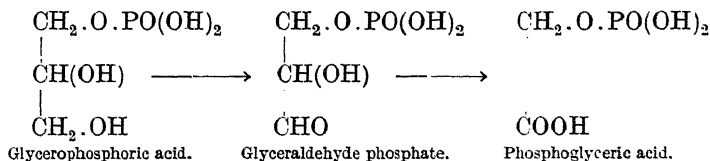


Cucumber seed is a good source of citric dehydrogenase, and, used with methylene blue as a hydrogen acceptor, forms a very delicate test for citric acid (Thunberg).

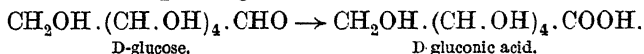
(6) **Alcohol dehydrogenase**, found in liver and kidney, dehydrogenates primary and secondary alcohols into the corresponding aldehyde or ketone. The process requires the presence of a special co-enzyme.



(7) **Glycerophosphate dehydrogenase**, from yeast, converts α -glycerophosphoric acid into glyceraldehyde phosphate, and subsequently into phosphoglyceric acid, all of which participate in glycolysis. Adenosine triphosphate is the co-enzyme of the system.



(8) **Glucose dehydrogenase**, found by D. Harrison in liver, oxidises glucose into the corresponding hexonic acid.



(9) **Hexose monophosphate dehydrogenase**, from yeast and red blood cells, converts the sugar ester into the corresponding phosphohexonic acid.

(10) **Triose phosphate dehydrogenase**, in yeast and muscle, oxidises triose phosphate to phosphoglycerate, and is part of the chemical mechanism in muscle contraction and in yeast fermentation (p. 291).

(11) **Hexose diphosphate dehydrogenase**, from muscle, liver, cucumber seed and other sources, activates fructose diphosphate.

These dehydrogenases activate simple substrate molecules derived from the hydrolysis or deamination of food materials. Other types of dehydrogenase activate the more complex compounds that function as hydrogen-carriers in respiration.

(12) **Co-enzyme dehydrogenases** (*diaphorases*) activate co-enzymes to effect indirect oxidation of the primary substrate by cytochrome or flavoprotein.

B. Oxidases catalyse oxidation of their substrate by free oxygen, and thus form the last member of a catalytic series. The substrate may be a food metabolite, a toxic end-product, or a hydrogen-carrier.

(1) **Cytochrome dehydrogenase**, or indophenol oxidase, accompanies cytochromes in tissues (p. 195).

(2) **Monophenol oxidase**, or tyrosinase (p. 226).

(3) **Polyphenol oxidase**, or laccase.

(4) **"Dopa" oxidase** (p. 309).

Only the first of these oxidases is of general importance in tissue respirations. The others represent enzymes concerned in specialised aspects of plant or animal metabolism.

C. Peroxidases occur freely in tissues, rich sources being spleen, lung, liver, seedling sprouts and root vegetables, especially the horse radish. Peroxidases catalyse the transfer of oxygen from peroxides to oxidisable substrates, without the liberation of free oxygen in the process. In the absence of a hydrogen acceptor, peroxidases do not decompose hydrogen peroxide (p. 227).

D. Catalase is almost universal in plant and animal tissues, a particularly rich source being horse liver, from which the enzyme has been obtained in crystalline form. Catalase converts hydrogen peroxide to water and free oxygen. Hydrogen peroxide is a toxic compound, and may arise in various biological oxidations. By means of a peroxidase system it may be employed to effect subsequent oxidations, or by means of catalase it may be removed rapidly.

The Respiratory Carriers

Only one type of enzyme, the aerobic oxidases, is able to oxidise the substrate directly by means of free oxygen, which acts as a hydrogen acceptor and is reduced to hydrogen peroxide. The liberated peroxide is either decomposed by catalase or used for secondary reactions.

The majority of tissue oxidations are complex, and require the presence of a chain of respiratory carriers that transport hydrogen

from the substrate-dehydrogenase complex to the activated oxygen. Unlike the respiratory enzymes, respiratory carriers are thermostable compounds capable of reversible oxidation-reduction. They are represented by :—

(1) *Ferroproteins*, or *respiratory hæmatins*; the respiration catalyst or “Atmungsferment” of Warburg, and the cytochromes of Keilin.

(2) *Flavoproteins*, or alloxazine proteins: the “yellow enzyme” of Warburg, the co-enzyme of D-amino acid oxidase.

(3) *Pyridine proteins*, conjugates of pyridine nucleotides: co-enzyme (co-enzyme I, or co-dehydrogenase I), co-enzyme II (co-dehydrogenase II).

(4) *Oxaloacetic acid*, in the dicarboxylic system of Szent-Györgyi.

(5) *Citric acid*, in the cycle of Krebs and Johnson.

(6) *Thiol compounds*: glutathione.

(7) *Ascorbic acid*, or vitamin C.

(8) *Thiamine*, aneurin or vitamin B₁, in co-carboxylase.

(9) *Miscellaneous respiratory pigments*, including adrenochrome, (an oxidation derivative of adrenaline) and pyocyanine.

The great majority of metabolites in animal tissues are oxidised through the intermediation of the ferroproteins and the co-enzymes.

(1) **Ferroproteins.**—From his work, begun in 1913, Warburg concluded that an organic iron compound, present as a micro-constituent of all tissues, is the primary factor in cell respiration, and operates by making free or atmospheric oxygen available for oxidising metabolites.

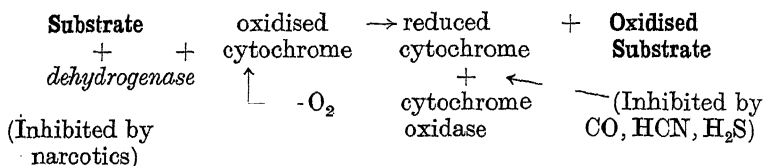
In support of this, he showed (i.) that iron occurs in concentrations of 10 γ to 100 γ per gm. of cell substance in all active tissues, and (ii.) that agents reacting with iron, such as HCN, CO and H₂S, are powerful inhibitors of tissue respiration. Cyanide in M/100 concentration inhibits about 90 per cent. of the total respiration of most tissues; the residual 10 per cent. is cyanide-stable, and due to the presence of flavoprotein carriers. Exceptional tissues are cardiac ventricle and diaphragm, with cyanide-stable respirations of 41 and 30 per cent. respectively.

Warburg concluded that his factor was a porphyrin compound, since (i.) tissues after exposure to CO display a spectrum resembling a CO-hæmatin, and (ii.) respiratory inhibition by CO is sensitive to light, being reversed by strong illumination in a manner similar to the photo-dissociation of carboxyhæmoglobin.

In 1925, Keilin showed that cytochromes occurred in all aerobic tissues, and possessed many characteristics of Warburg's factor. These cytochromes are three in number (p. 195), and are able to exist in oxidised or reduced form. Oxidised cytochrome is capable

of oxidising many, but not all, tissue metabolites; and, in turn, is reoxidised by molecular oxygen in presence of the enzyme *cytochrome oxidase* (indophenol oxidase), which accompanies cytochrome.

Reoxidation of cytochrome is inhibited by HCN, CO and H₂S in a manner similar to the inhibition of Warburg's factor, and it is concluded that the cytochromes and the Warburg factor are similar in character, and form part of the system whereby molecular oxygen becomes available for tissue respiration. According to Warburg (1934) all four compounds form a chain, the members of which are alternately oxidised to ferric or reduced to ferrous state, thus transmitting an oxidation potential to the first member of the carrier series which accepts the hydrogen from the substrate activated by dehydrogenase.



(2) **Flavoproteins.**—In 1932, Warburg and Christian isolated an enzyme from yeast, which, owing to its colour they named the "yellow enzyme" (gelbes Ferment). In presence of a co-enzyme and a dehydrogenase (Zwischenferment) the yellow enzyme catalysed the oxidation of hexosemonophosphate (Robison's ester) to phosphogluconic acid by free oxygen. The co-enzyme of the system differed from co-enzyme in having an additional phosphoric radicle (p. 259). Subsequently it was shown that the cyanide-stable respiratory mechanism in yeast and other tissues included the yellow enzyme as a necessary component, and its importance in tissue respiration was recognised. The yellow enzyme is an example of a *flavoprotein*, or conjugated protein in which the prosthetic group contains a flavin.

In the yellow enzyme, the prosthetic group is the phosphate of riboflavin, or vitamin B₂ (p. 256). The flavoprotein carrier differs from hematin carriers in three respects: (i.) it does not contain iron and is not inhibited by CO, HCN and H₂S; (ii.) it requires the presence of an additional carrier, co-enzyme II; (iii.) it is capable of being reoxidised by free oxygen without the aid of an oxidase. At the same time, flavoprotein can work in conjunction with cytochrome to form a system containing three successive respiratory carriers.

(3) **Pyridine Nucleotides.**—These are represented by the co-enzymes I and II, also termed the co-dehydrogenases or co-dehydrases. They are compounds assembled from nicotinic amide. Their constitution may be represented diagrammatically as :—

Co-enzyme I, nicotinic amide-ribose-(phosphoric acid)₂-ribose-adenine.

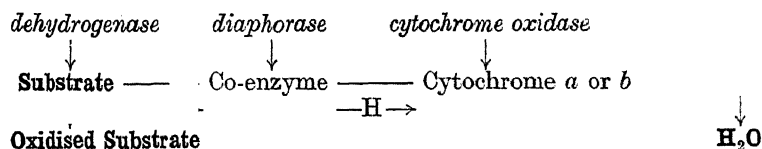
Co-enzyme II, nicotinic amide-ribose-(phosphoric acid)₃-ribose-adenine.

The structural formula is given on p. 259.

By undergoing alternate oxidation and reduction in the nicotinic nucleus, the co-enzymes act as hydrogen carriers in oxidation systems, and form a necessary link in the complicated chains of tissue activity. Oxidation of the reduced co-enzyme is catalysed by a special enzyme, *diaphorase*, a flavoprotein present in most animal tissues.

The effect of this oxidation is to transfer hydrogen from the co-enzyme to the next carrier in the series, namely, cytochrome *a* or *b*. The reduced cytochrome is then reoxidised by free oxygen, with the formation of water, the end-product of the metabolic process.

In the biological reaction, the substrate is oxidised by the transfer of hydrogen to successive carriers, the last of which reacts with oxygen.

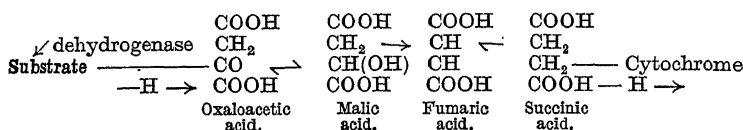


Co-enzyme I (co-zymase) acts as a hydrogen carrier in sugar fermentation and in muscle contraction, and various tissue oxidations.

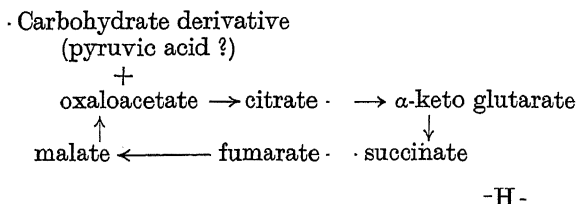
Co-enzyme II acts as a hydrogen carrier in the dehydrogenation of glutamic acid, hexosemonophosphate and glucose. All the other known systems that require a co-dehydrogenase employ co-enzyme I.

(4) **The Oxaloacetic System** (Szent-Györgyi, 1937).—The wide distribution of the three enzymes, succinic and malic dehydrogenase, and fumarase, suggests that they and their respective substrates participate in many tissue respirations. Addition of malonic acid, which inhibits succinic acid oxidation, leads to an almost complete

suppression of the respiratory activity of a tissue, whereas addition of fumaric acid greatly increases respiration. From these observations, Szent-Györgyi concluded that an intermediate system, consisting of the four C_4 dicarboxylic acids, functions as a hydrogen carrier between the primary substrate and cytochrome.

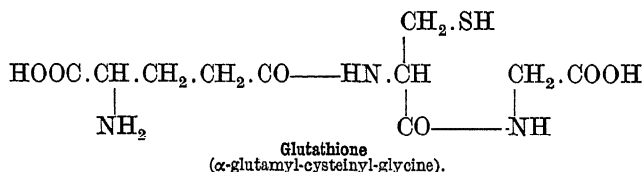


(5) **The Citric Cycle** (Krebs and Johnson, 1937).—Citric acid, like succinic acid, may stimulate carbohydrate oxidation in muscle, and can arise from any one of the four acids in the oxaloacetic system. Krebs and Johnson believe that the oxaloacetic system is part of a cycle in which the order is :—



These systems are of interest in connection with the failure of the carbohydrate utilisation mechanism in diabetes, and should have a practical application in clinical treatment.

(6) **Glutathione** is a natural tripeptide derived from glutamic acid, cysteine, and glycine. It is very widely distributed in animal tissues and forms part of the oxidation-reduction equipment.



Sources.—Glutathione was isolated in 1921 from yeast, muscle, and mammalian liver by Hopkins, and identified as the compound responsible for Mörner's cysteine reaction—the development of a violet colour when the tissue is treated with sodium nitroprusside (nitroferrocyanide) and ammonium hydroxide. The reaction is specific for the *thiol* or *sulphydryl* group, —SH , and is given by most

animal tissues. Glutathione is almost universally distributed in the animal kingdom, and also occurs in yeasts, fungi, and bacteria. It is present in blood corpuscles, but absent from blood serum.

Estimated glutathione percentages of fresh tissues are: yeast, 0.15-0.2; rabbit liver, 0.18-0.35; skeletal muscle, 0.045; kidney, 0.15; blood plasma, 0.0.

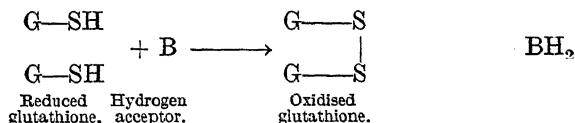
Glutathione may be obtained by the rapid extraction of pressed baker's yeast with 0.1 per cent. acetic acid, and subsequent precipitation with neutral lead acetate followed by mercuric sulphate. After removal of the metals by H_2S , the peptide is selectively precipitated by addition of cuprous oxide (Hopkins, 1929). The tissue content of glutathione may be estimated by titrating with 0.001 N iodine, the solution that is obtained by exhaustive extraction of the material with 10 per cent. trichloroacetic acid until the extract no longer gives a violet colour with nitroprusside and ammonia. The method is not specific, any other labile thiol compound reacts in a similar fashion.

It is concluded, however, that glutathione is the chief thiol compound in tissues; cysteine, which is next in importance, is probably less than one-twentieth the value of glutathione. Glutathione is a colourless, crystalline solid. It is very soluble in water and in dilute alcohol. These solutions are unstable, and if slightly alkaline, readily oxidise to form the double sulphide:—



The change is analogous to the conversion of cysteine into cystine. This auto-oxidation of glutathione is attributed by Voegtlin (1931) to the presence of minute traces of copper.

Significance of Glutathione.—The outstanding biochemical property of glutathione is its ability to assume either of two forms under conditions that obtain in living tissues:—



Animal tissues contain systems that vigorously oxidise the reduced glutathione, and systems that vigorously reduce the oxidised glutathione by means of molecular oxygen; hence the tripeptide is capable of acting as an oxygen carrier.

Tissue glutathione is chiefly in the reduced form, as shown by the positive nitroprusside test. The reducing agent is unknown, and cannot be traced to one of the familiar dehydrase systems (succin-oxidase, xanthine-oxidase, lactic-oxidase, citric-oxidase), and the

high thermostability of the agent indicates that it is not an enzyme. Reduced glutathione in neutral or slightly alkaline solution is auto-oxidisable, depending on the presence of copper or iron. Since both these metals are invariably present in living cells as micro-constituents it is possible that their exposure or concealment may be a means whereby the oxygen uptake by glutathione is determined. $G-SH$ in acid solutions of about pH 4 is not auto-oxidisable, even in presence of added copper or iron. Yet on the addition of an acid emulsion of unsaturated fats or fatty acids there is a steady and continuous uptake of oxygen that is far in excess of the amount that would be required to convert all the $G-SH$ into $G-S-S-G$, showing that the tripeptide must have catalysed the aerobic oxidation of the lipide.

The almost universal distribution of glutathione shows it must be of great importance. For example, it is necessary for the hydration of methyl glyoxal to lactic acid by the enzyme glyoxalase, and thus may form part of the mechanism of carbohydrate fermentation.

(7) **Ascorbic acid**, vitamin C, can act as an oxygen carrier for oxidation of glutathione (Hopkins and Morgan, 1936). Ascorbic acid is oxidised by the enzyme *ascorbic oxidase*, obtained from plant tissue, where the acid presumably forms part of a respiration system. Oxidised ascorbic acid is rapidly reduced by glutathione, and thus is protected against oxidation by free oxygen or by ascorbic oxidase. Ascorbic acid is such a powerful reducing agent that it must be regarded as a potential oxygen acceptor in many forms of metabolism.

(8) **Thiamine**, aneurin or vitamin B_1 is directly concerned with intermediate carbohydrate metabolism, and the extensive work of R. Peters has shown that in the form of its diphosphoric ester (co-carboxylase), it works in conjunction with the dicarboxylic acid system of Szent-Györgyi in bringing about the characteristic oxidation of pyruvate in brain tissue.

Co-carboxylase, the pyrophosphoric ester of vitamin B_1 , is a characteristic constituent of yeast, where it co-acts with an enzyme in the decarboxylation of pyruvic acid to acetaldehyde and carbon dioxide during fermentation (p. 298). In the organism, vitamin B_1 circulates in free form or as the monophosphate, and is converted to the co-enzyme locally within the tissues. The co-carboxylase in the blood is restricted entirely to the blood cells.

Oxidation Inhibitors

Two classes of compounds inhibit cell respiration: (1) cyanide, hydrogen sulphide, sodium azide, and carbon monoxide in very low

concentrations act chiefly by preventing cytochrome oxidation by cytochrome oxidase ; (2) narcotics, such as urethane, act in high concentrations by inhibiting dehydrogenase systems from activating the primary substrates. Oxidation-inhibition and narcosis are distinct but not necessarily unrelated phenomena, since narcosis can be induced in anaerobic cells, and narcotics do not inhibit the oxidation of various metabolites by brain cortex, with the important exceptions of glucose, lactate and pyruvate. According to Quastel, narcotics are adsorbed from the cerebro-spinal fluid at specific areas in the nervous system, and by inhibiting local oxidation of glucose, lactate or pyruvate, lessen the supply of energy available for the functional activity of the cells.

Classification of Oxidases and Dehydrogenases (Elvehjem, 1939).

I. Oxidases.

- (1) Presumably activate oxygen.
- (2) Do not reduce dyes.
- (3) Do not act in absence of oxygen.
- (4) Catalyse direct reaction of metabolites with oxygen.
- (5) Produce H_2O_2 .
- (6) Inhibited by cyanide.
- (7) Require neither co-enzyme nor cytochrome.

Examples : (a) Cytochrome oxidase (indophenol oxidase).

(b) Tyrosinase (monophenol oxidase).

(c) "Dopa" oxidase (dihydroxyphenylalanine oxidase).

(d) Polyphenol oxidase.

(e) Ascorbic acid oxidase.

II. Peroxidases.

- (1) Oxidise substrates by means of H_2O_2 .
- (2) Inhibited by cyanide.

III. Catalase.

- (1) Decomposes H_2O_2 to O_2 and H_2O .
- (2) Inhibited by cyanide.
- (3) May have peroxidase activity.

IV. Aerobic Dehydrogenases.

- (1) Activate hydrogen of metabolites.
- (2) Reduce dyes.
- (3) Act in absence of oxygen when suitable dyes are present.
- (4) Catalyse direct reaction between metabolites and oxygen.
- (5) Produce peroxide in presence of oxygen.
- (6) May or may not be inhibited by cyanide.

(7) Require neither co-enzyme nor cytochrome.

Examples : (a) Xanthine oxidase (Schardinger enzyme).

(b) Tyramine dehydrogenase.

(c) Uricase.

V. Anaerobic Dehydrogenases (classified according to the first carrier).

(1) Activate hydrogen of the metabolites.

(2) Catalyse reaction between metabolites and carriers.

A. Cytochrome-linked Dehydrogenases :

(a) Succinic dehydrogenase.

(b) α -glycerophosphate dehydrogenase.

B. Co-enzyme I-linked Dehydrogenases :

(a) Lactic dehydrogenase.

(b) Malic dehydrogenase.

(c) β -hydroxybutyric dehydrogenase.

(d) Citric dehydrogenase.

(e) Glucose dehydrogenase.

(f) Alcohol dehydrogenase.

(g) Aldehyde mutase.

(h) Triose phosphate dehydrogenase.

(i) Dihydroxyacetone dehydrogenase.

(j) L(+)-glutamic acid dehydrogenase.

C. Co-enzyme II-linked Dehydrogenases :

(a) Glucose dehydrogenase.

(b) Hexose monophosphate dehydrogenase.

D. Unclassified :

(a) Choline dehydrogenase.

(b) α -keto acid dehydrogenases.

(c) Fatty acid dehydrogenases.

(d) Histaminase.

Summary of the Dehydrogenase—Carrier Systems

(Classification according to D. E. Green)

S = primary substrate for oxidation ; *SO* = oxidised substrate.

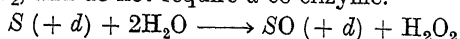
d = primary dehydrogenase ; *cy.d* = cytochrome dehydrogenase (or oxidase).

Cy = reduced cytochrome ; *CyO* = oxidised cytochrome.

Cd = reduced co-dehydrogenase (co-enzyme) ; *CdO* = oxidised co-dehydrogenase.

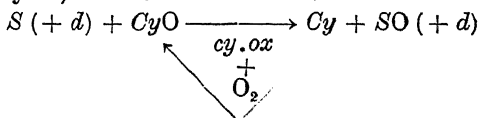
F = reduced flavoprotein ; *FO* = oxidised flavoprotein.

(i.) *Aerobic dehydrogenases* : react directly with molecular oxygen, produce H_2O_2 , and do not require a co-enzyme.



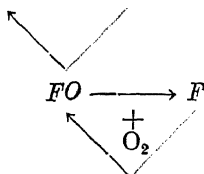
(ii.) *Simple cytochrome systems*: react with molecular oxygen through the intermediary of cytochrome activated by cytochrome oxidase. Require neither flavoprotein nor co-enzyme.

E.g.—*Dehydrogenases* of α -glycerophosphate, succinate, and (for the yeast enzyme) lactate.

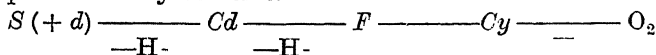


(iii.) *Simple co-dehydrogenase systems*: react with molecular oxygen through the successive intermediaries of co-dehydrogenase I or II and flavoprotein. Do not require cytochrome.

E.g.—(a) Co-dehydrogenase I systems: *dehydrogenases* of malate, fumarate, glucose (liver), and lactate (muscle). (b) Co-dehydrogenase II systems: *dehydrogenase* of hexose phosphate (yeast and red blood cells).



(iv.) *Cytochrome-flavoprotein systems*: react with molecular oxygen through the successive intermediaries of co-dehydrogenase, flavoprotein and cytochrome.



OXIDATION-REDUCTION POTENTIAL

The majority of biochemical decompositions are oxidative in nature, and the tendency for a reactant to undergo oxidation or reduction can be measured in terms of a potential. Every oxidation involves a corresponding reduction of some other reactant, and may be regarded as an interaction between an *oxygen donator* and an *oxygen acceptor*, owing to difference in oxidation-reduction potential.

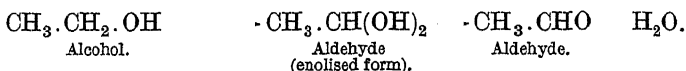
These potentials may be determined electrometrically by a method analogous to that adopted in measuring H-ion potentials, but there are difficulties in obtaining significant results.

For example, many oxidations are highly sensitive to changes in pH, and are enormously affected by the presence of traces of catalytic metals, notably iron and copper. Also, the method is

based on the assumption that the reaction is reversible. Now, biological metabolism is typically irreversible. It is not possible by feeding an animal with carbon dioxide, water, and urea to persuade it to reconvert these end-products into protein.

Hence, instead of trying to determine the oxidation-reduction, or *redox* potentials of living tissues, investigators have sought to isolate simple chemical systems capable of undergoing reversible oxidative changes, and to measure their potential under conditions similar to those occurring in the parent tissue.

The Electrochemical Theory of Oxidation-Reduction.—Formerly the phenomena of oxidation and reduction were regarded as direct additions of oxygen or hydrogen. Thus, the conversion of alcohol into aldehyde was explained by the assumed formation of an intermediate dihydroxy compound :—



Elements with variable valency, such as iron and copper, however, can be oxidised or reduced without the acquisition or loss of oxygen ; and the concept was extended to include oxidative *increase* or reductive *decrease in valency*. The valency of an element is measured by its ability to gain or lose electrons. When an atom of ferrous iron, Fe^{++} , passes into the ferric state, Fe^{+++} , there is an increase in the positive charge due to loss of the negatively-charged electron ; and when ferric iron becomes ferrous iron there is a gain of one electron, and a consequent decrease in the positive charge. That is to say, the oxidation of an element involves the loss of one or more electrons, and the reduction of an element involves the gain of one or more electrons.

When hydrogen is oxidised to form water, each hydrogen atom loses an electron, and each oxygen atom is reduced by the gain of two electrons. The transfer of electrons in oxidation-reduction reactions is from the reducing agent to the oxidising agent.

A *reducing agent* is a substance capable of donating electrons. An *oxidising agent* is a substance capable of accepting electrons. When a reducing agent and an oxidising agent are mixed in one solution, electron exchange takes place directly between the two reactants. If, however, the reactants are in separate containers connected by an inverted U-tube with an electrolyte, such as KCl, nothing happens until an external circuit is established by means of a conductor with an electrode immersed in each container.

Under these conditions, the electrons from the reducing agent are taken up by its electrode and travel along the conductor to the electrode immersed in the oxidising agent, and the consequence

is the same as if the reactants had been mixed in the same container. This arrangement constitutes an electrolytic unit for the production of a current by a chemical reaction, and is exemplified by the various types of primary cells and "dry batteries" in common use.

Electrode Potential.—The potential difference set up between the two solutions in the oxidation-reduction unit is determined by the electron-donating tendency or "electron-fugacity" on the part of the reducing agent. This can be measured electrometrically by means of an inert electrode immersed in a solution of the reducing agent, the other solution being replaced by a hydrogen-electrode cell containing a buffer solution at the same pH as the reducing agent. Under these conditions, any potential difference between the two electrodes will be due to differences in electron concentration and not H-ion concentration. The potential difference, when referred to the standard hydrogen electrode and measured in volts is termed the *reduction potential*, E , or E_H .

The value of E depends both on the concentration of the reducing agent and the extent to which it has undergone oxidation owing to loss of electrons; consequently, the zero value, E_0 , is obtained when the reducing agent has come into equilibrium with its oxidised form, and both are present in equal concentration. Adopting the logarithmic notation similar to that used in expressing H-ion concentrations,

$$\frac{[\text{Oxidised form}]}{[\text{Reduced form}]}$$

where k is a constant depending on the absolute temperature. For a quinhydrone system at a uniform pH and at 30° C., the value of k is 0.03. Just as pH is determined by a balance between H-ions and OH-ions, E is determined by a balance between the oxidised and reduced forms of the reactant in the solution. The values of E_0 for a number of oxidation-reduction systems have been estimated, and a scale of voltage intensities can be prepared, the higher representatives of which will oxidise the lower representatives.

Oxidation-Reduction Potentials

System.	Solvent.	Temperature.	E_0 .
Mn \cdots /Mn \cdots	15N H ₂ SO ₄	12°	+ 1.511
Fe \cdots /Fe \cdots	0.1N HCl	25°	+ 0.743
Quinhydrone/hydroquinone	0.1N HCl	18°	+ 0.618
I ₂ /I \cdots	H ₂ O	—	+ 0.54
Cu \cdots /Cu \cdots	H ₂ O	—	+ 0.18
V \cdots /V \cdots	N H ₂ SO ₄	18°	— 0.204
Sn \cdots /Sn \cdots	N HCl	25°	— 0.426
Sn \cdots /Sn \cdots	0.6N NaOH	18°	— 0.854

Whether a substance is an oxidising or a reducing agent is determined by its ability to accept or donate electrons. Systems with a high positive value of E_0 are regarded as oxidising agents since they can accept electrons readily; and systems with a high negative value of E_0 are generally described as reducing agents.

Oxidation-Reduction Indicators.—*Redox Indicators.*—Many dye-stuffs and natural pigments are converted to colourless *leuco* compounds on reduction. The change is reversible, and the colour intensity of the compound is determined by proportions of the components present.

Methylene blue is a familiar example of a pigment readily bleached by reducing agents. Its sensitivity varies greatly with the hydrogen ion concentration, as shown by the value of its E_0 at 30°, which ranges from +0.1 (pH 5.0) to -0.5 (pH 9.0). Under constant conditions of temperature and pH the degree of reduction can be found from the colour intensity of the mixture.

Colour Intensity of Methylene Blue at pH 7.0 and 30° C.

E_0	-0.01	-0.003	+0.011.	+0.025.	+0.062 volts.
Colour . .	none	intermediate shade			deep blue
Oxidised form .	0.1%	25%	50%	75%	98%
Leuco form .	99.9%	75%	50%	25%	2%

A series of oxidation-reduction indicators of varying degrees of sensitivity has been prepared, and may be applied to the detection and estimation of biological reactants.

Important examples are: (i.) The use of methylene blue as a hydrogen acceptor in dehydrogenase systems. (ii.) The *nadi* reagent, which led to the elucidation of the Warburg-Keilin system. (iii.) The application of methylene blue as an internal indicator in sugar estimation by Lane and Eynon. (iv.) The estimation of ascorbic acid by titration with dichlor-indophenol.

Summary.—As Szent-Györgyi has pointed out (*Harvey Lectures*, 1938-39), the primary fuel of life is hydrogen. All organic food is essentially but a fixed form of hydrogen, and all the energy that supports life is derived from the oxidation of hydrogen to water. This is accomplished by the cytochrome system of Keilin and the Warburg catalyst. One at least of the cytochromes contains iron. This iron is alternately oxidised and reduced. Ferrous

iron contains one electron more than ferric iron, and the transference of this electron activates the series of interlinked reactions that constitute the respiratory process.

GENERAL REFERENCES

- BARRON, E. S. G. (1939), "Cellular oxidation systems." *Physiol. Rev.*, **19**, 184.
- BEUTNER, R. (1933), "Physical Chemistry of Living Tissues." London.
- BIGWOOD, E. J. (1936), "Le mécanisme de la respiration cellulaire." Paris.
- DAKIN, H. D. (1922), "Oxidations and Reductions in the Animal Body." Monographs on Biochemistry. London.
- DIXON, M. (1929). "Oxidation mechanism in animal tissue." *Biol. Rev.*, **4**, 352.
- ELVEHJEM, C. A., and P. W. WILSON (1939), "Respiratory Enzymes." Minneapolis.
- KEILIN, M. D. (1936), "Intracellular respiration." *Bul. Soc. Chim. Biol.*, **18**, 96.
- MELDRUM, N. U. (1934), "Cellular Respiration." London.
- QUASTEL, J. H. (1939), "Respiration in the central nervous system." *Physiol. Rev.*, **19**, 135.
- ROCHE, J. (1936), "La biochimie générale et comparée des pigments respiratoires." Paris.
- SZENT-GYÖRGYI, A. (1937), "Studies on Biological Oxidation." Leipzig.
- WIELAND, H. (1932), "The Mechanism of Oxidation." Oxford.

CHAPTER XX

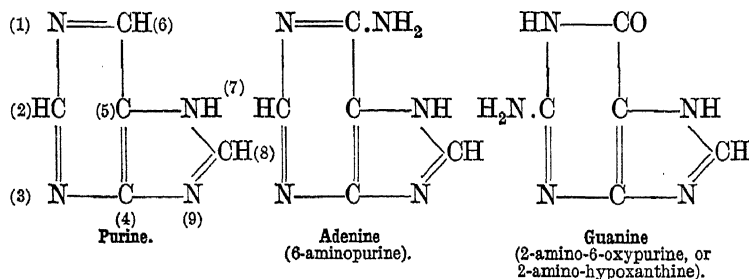
PURINES AND PYRIMIDINES

Purines.—The biological purines are simple hydroxy, amino, or methyl derivatives of a parent purine ring, which does not occur free in nature. Hydroxy purines, *hypoxanthine* and *xanthine*, occur in tissues and tissue fluids of many animals; *uric acid*, a trihydroxy purine, is the chief nitrogenous excretory product of birds and snakes, and is invariably present in mammalian urine.

Amino purines, represented by *adenine* and *guanine*, are components of nucleic acid and the nucleosides. Methyl purines are the characteristic alkaloids of tea, coffee, and cocoa.

Sources of the Purines.—The hydroxy and amino purines are derived chiefly from the nucleoproteins of the diet, although there is evidence that the organism can synthesise the purine ring from the amino acid histidine. Nucleoproteins are compound proteins, the non-protein being nucleic acid, which is liberated during alimentary digestion. Nucleic acid is composed of four nucleotide units, each of which is a phosphoric ester of a nucleoside, or compound of sugar and a nucleopurine or a pyrimidine (p. 130).

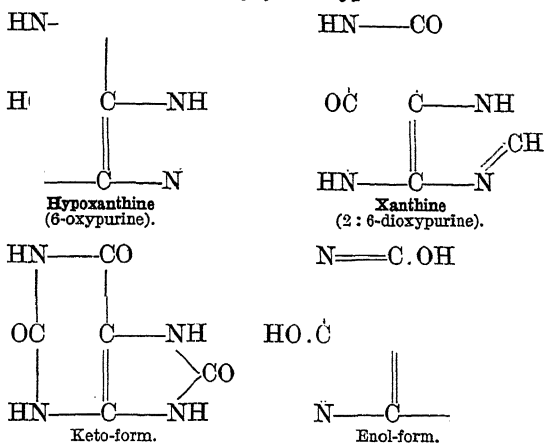
Aminopurines, or Nucleopurines



Adenine, $\text{C}_5\text{H}_5\text{N}_5$, the simpler of the nucleopurines, is widely distributed in plant and animal nucleoprotein, in the mononucleoside *adenosine*, found in animal tissues, the adenylyl triphosphate of muscle, and as mononucleosides of beet, and human blood and urine. Adenine occurs in colourless needles that are slightly soluble in cold water, but freely soluble in acids or alkalis. Large amounts of adenine may appear in the urine in conditions of leukæmia. On deamination by enzymes or by nitrous acid, adenine is converted into the corresponding 6-oxypurine, *hypoxanthine*.

Guanine, $C_5H_5N_5O$, is widespread as a nucleopurine, and often accompanies hypoxanthine in plants. It occurs in guano, in muscle, in the juice of the beet, in many leguminous seeds, and as an articular deposit in the joints of swine suffering from guanine gout. It resembles adenine, but is a stronger base. On oxidation, it gives rise to *guanidine*, $H_2H-C(:NH)-NH_2$, which serves to distinguish it from adenine and all other purines. On deamination, guanine is converted into the corresponding 2:6-dioxypurine, *xanthine*.

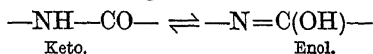
Hydroxy-, or Oxypurines



Uric Acid

(2:6:8-trioxypurine).

Each of these purines can exist either in keto or in enol form, owing to the tautomeric change:—



Hypoxanthine, $C_5H_4N_4O$, has been obtained chiefly from nucleotides. It is present in extracts of glandular and muscle tissue, in fish sperm and bone marrow, and, in traces, in milk and urine. Urinary hypoxanthine is greatly increased in leukaemia. It occurs in microcrystals of low solubility in water and organic solvents, but dissolves in acids or alkalis to form salts. On oxidation, it is converted into xanthine.

Xanthine, $C_5H_4N_4O_2$, is present, alone or combined, in many animal extracts, and is one of the minor constituents in mammalian urine, and in guano. Like hypoxanthine, it is chiefly of interest in being a deamination product of an amino purine, and an obligatory intermediate product in uric acid metabolism.

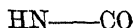
Uric acid, $C_5H_4N_4O_3$, the most important of the oxidised purines, is the chief end-product of purine metabolism in man and the higher apes. It is the chief end-product of protein metabolism in uricotelic animals, birds and snakes, and some invertebrates. It is the least soluble of all the forms in which nitrogen is excreted, and appears in calculi, articular deposits, and urinary sediments.

Distribution of Uric Acid in the Human Body.—Blood contains 1–5 mg. per 100 ml. The value is raised typically in gout, lead poisoning, leukæmia, and renal inefficiency. The uric acid content of the tissues is usually lower than that of the blood, unless in a region of active purine metabolism.

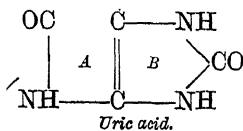
Urine contains about 40–150 mg. uric acid per 100 ml., representing the average daily excretion of 0.5–1.5 gm. The value is extremely variable, depending on dietetic and individual conditions.

Properties of Uric Acid.—The acid is colourless, odourless, and tasteless. Its solubility in water is extremely low, being 1 : 39,000 at 18° C., and 1 : 15,500 at body temperature (37° C.). It dissolves readily in alkalis with the formation of (i.) acid salts, or monobasic urates, and (ii.) neutral salts, or dibasic urates. Biological urates are acid urates; the neutral urates are only stable under conditions of alkalinity not found in the organism. Although uric acid is not a true organic acid, since it contains no carboxyl groups, its power of salt formation is due to enolisation of the three hydroxyl groups as displayed in the form $C_5HN_4(OH)_3$.

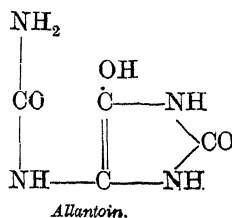
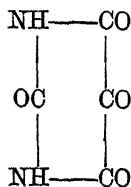
Oxidation of Uric Acid.—Oxidation of uric acid in alkaline solution opens up the pyrimidine ring, producing *allantoin*. Oxidation in acid solution opens up the iminazole ring, producing *alloxan*.



A = pyrimidine ring



B = iminazole ring



Allantoin is not a purine, since the characteristic double ring has been opened, but is of importance since it represents the end-product of purine metabolism in many animals. It occurs in the allantoinic fluid of herbivora, and in the urine of herbivora and carnivora in the following average concentrations; expressed in grams per litre: calf, 5-6; cow, 2-2.5; sheep, horse, pig, rabbit, 0.7-1.8; dog, 1.9-2.6. The amount excreted daily by the cow is 20-30 gm. Only small and variable traces are found in human urine; its place being taken by its precursor uric acid.

Allantoin is much more soluble than uric acid, the solubility in water being 1:160 at 20° C. For this reason it is much more desirable as an end-product of purine metabolism, since it does not form calculi.

Allantoin, like urea, gives a colour with Ehrlich's reagent (p. 149), and with diacetyl monoxime in acid solution (p. 384).

Alloxan is chiefly of chemical interest in regard to the structure of uric acid. It does not participate in any known biological reaction.

Detection of Uric Acid.—(i.) *Reduction Tests.*—Uric acid in alkaline solution can reduce silver salts (Schiff's test), copper salts, phosphomolybdates and phosphotungstates. The last of these reactions is the basis of Folin's method for detection and estimation of the acid.

(ii.) *Murexide Test.*—This reaction is very useful for detecting uric acid in solids, such as powdered calculi. The solid is moistened with concentrated nitric acid, and dried in a dish on a water bath. The warm residue has a brilliant orange-carmine colour if uric acid be present. Addition of an alkali changes the colour to purple (ammonium hydroxide), or violet (sodium hydroxide). Other purines, notably xanthine, guanine, and caffeine, give somewhat similar colours, but they do not occur in urinary precipitates and calculi. The test has been modified by Milroy so as to be specific for uric acid.

Natural Purine Derivatives

Purines occur as (i.) nucleosides, (ii.) mononucleotides, and (iii.) polynucleotides, or nucleic acids. Nucleosides are glycosides of a purine or pyrimidine base. Nucleotides are phosphoric esters of nucleosides, in which the phosphoric radicle is joined to the third or fifth carbon in the sugar residue.

(i.) *Purine Nucleosides* :—

Adenosine, 7-adenine-D-ribose.

Guanosine, 7-guanine-D-ribose.

Inosine, 7-hypoxanthine-D-ribose.

(ii.) *Purine Mononucleotides* :—

Adenylic acid (from muscle), adenine-5-phosphoriboside.
 Adenylic acid (from yeast), adenine-3-phosphoriboside.
 Inosinic acid, hypoxanthine-5-phosphoriboside.
 Guanylic acid, guanosine phosphate.

(iii.) *Mononucleotide Derivatives* :—

Adenosine triphosphoric acid (adenyl pyrophosphate).
 Adenosine diphosphoric acid (adenyl phosphate).
 Co-enzyme I.
 Co-enzyme II.
 Co-enzyme of D-amino acid oxidase.

(iv.) *Polynucleotides* :—

Thymo-nucleic acid, desoxyribose nucleic acid.
 Yeast-nucleic acid, ribose nucleic acid.

The nucleosides are prepared by alkaline hydrolysis of the parent nucleotides, and rarely occur in a free state.

Mononucleotides

Adenylic acid, now called "muscle adenylic acid" to distinguish it from adenylic acid obtained from yeast, is widely distributed in animal tissue, and ranks along with histamine and acetyl choline as a powerful vaso-dilator. On deamination it is converted to the much less active inosinic acid. Adenylic diphosphate (or pyrophosphate) is found in muscle where it acts as a donator of phosphoric acid in the contraction process (p. 291), becoming degraded to adenyl phosphate during the change.

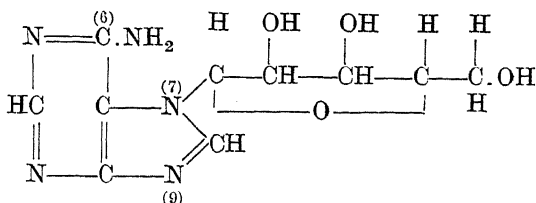
Co-enzyme I is a diphospho-pyridine nucleotide of adenine. Co-enzyme II (co-zymase) is a triphospho-pyridine nucleotide of adenine. Both co-enzymes act as hydrogen carriers in respiration (pp. 259, 330). The co-enzyme of D-amino acid oxidase is a diphospho-riboflavin nucleotide of adenine.

Polynucleotides

Nucleic acids occur in union with protamines or histones as the nucleo-proteins found in all plants and animals. They are substances of high molecular weight, the simplest unit being a tetranucleotide containing four different basic residues (adenine, guanine, cytosine, and thymine or uracil). On hydrolysis, a tetranucleotide yields four different nucleotides, each of which is changed into a nucleoside by alkaline hydrolysis. The sugar residue in the nucleic acid depends on the type of acid, and may be D-ribose or 2-desoxyribose. From these facts, provisional type formulæ have been assigned to the nucleic acids (p. 130).

Feulgen's Test for Nucleic Acid.—The material is hydrolysed with N/10 HCl at 60° C. for four minutes, and then treated with Schiff's aldehyde reagent (a 1 per cent. solution of rosaniline decolorised with SO₂), a red colour develops if animal or thymo-nucleic acid be present. The test depends on the presence of desoxyribose.

Structure of the Nucleosides.—The constitution assigned to the important nucleotides is given elsewhere in connection with their functions. The formula of a typical nucleoside is shown in adenosine. There is some uncertainty as to the point of attachment of the ribose group, and it is sometimes represented in union with the lower nitrogen atom (9) instead of the upper (7).



Adenosine

Phosphorylation of the terminal $-\text{CH}_2\cdot\text{OH}$ in the ribose group produces muscle adenylic acid, from which in turn are derived adenylic phosphate and adenylic pyrophosphate (adenosine triphosphate).

According to Conway (1938), adenosine and adenylic acid are the chief sources of the ammonia latent in blood, and are deaminated by plasma and tissue deaminases, with loss of the free amino group at position (6).

Plant Purines.—While all plants are able to synthesise purines, many do so in great excess of their nuclear requirements.

Purine Content of Plants

Expressed as mg. Uric Acid per 100 gm. Fresh Edible Material

Plant.	Uric Acid.	Plant.	Uric Acid.
Lentil . . .	160	Mushroom . .	15-30
Green pea . .	54-80	Celery . . .	15
Spinach . . .	70	Radish . . .	15
Kidney bean .	51	French bean .	6
Cauliflower .	24	Lettuce . . .	6
Asparagus . .	24	Potato . . .	6

Although these average values only refer to the edible portions of the plant, and do not discriminate between the various forms of

purine present, they indicate the existence of a group of vegetables rich in purine, and represented by the *Leguminosæ*. In addition, another class exists rich in methylated purines, and represented by tea and coffee, and other natural sources of the purine alkaloids.

Transformation and Degradation of Purine in Animals.—Animals other than birds and reptiles excrete their waste protein nitrogen in the urine, and are said to be *ureotelic*. In ureotelic animals, purine metabolism proceeds along independent lines. The purines of the diet, chiefly nucleotides and nucleosides liberated from nucleoproteins, are resolved into their constituent amino purines by the enzymes of the alimentary tract and mucosa. The amino purines are absorbed into the portal system, and if not utilised, are deaminated by the appropriate enzymes, adenase and guanase, found in the liver.

Adenine is converted into hypoxanthine, and guanine is converted into xanthine. Subsequently, by the action of xanthine oxidase both hypoxanthine and xanthine are converted into uric acid. Here the transformation stops in man, the higher apes, the Dalmatian dog, and the birds and reptiles; in consequence, uric acid is the characteristic end-product of purine metabolism. Most of the higher animals, however, are able to oxidise uric acid, and by opening part of the purine ring convert it into the more much soluble compound, allantoin, which is readily eliminated as a urinary solute.

Summary of Purine Transformation in Animals

Alimentary tract	<p>Nucleoprotein</p> <p>↓</p> <p>Nucleic acid</p> <p>↓</p> <p>Nucleotide</p> <p>↓</p> <p>Nucleosides</p>
Liver and other tissues	<p>Adenine Guanine</p> <p>↓ ↓</p> <p>Hypoxanthine Xanthine</p> <p>↓ ↓</p> <p>Allantoin Uric acid</p> <p>←</p>
Urine	

Enzymes concerned in Purine Metabolism.—(i.) **Adenase**, or adenine deaminase, is relatively rare. It is found in the spleen and

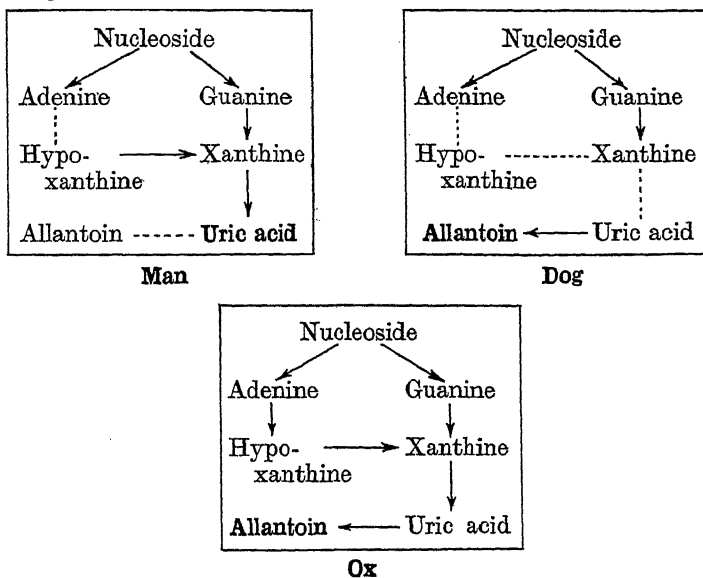
liver of the pig, and in the liver of the ox, but is absent from the liver of man, the dog, and the rabbit.

(ii.) **Guanase**, or guanine deaminase, is widely distributed, and is present in the liver and other tissues of most mammals, the pig being a remarkable exception.

(iii.) **Xanthine oxidase** catalyses the oxidation of both hypoxanthine and xanthine to uric acid, and also attacks adenine. It is found in the liver of many mammals, but not in the dog or the rat, indicating that in these animals uric acid must have an extra-hepatic source. Xanthine oxidase is a constant constituent of cow's milk.

(iv.) **Uricase**, or uric acid oxidase, is claimed to occur in the livers of all mammals except man and the higher apes. It is a purino-clastic enzyme, and converts uric acid into the non-purine allantoin.

Diagram of enzyme distribution in mammalian tissue



The continuous line indicates that the appropriate enzyme is present in the liver; the dotted line shows that it is absent. End-products of metabolism are in heavy type.

The diagram is based on the work of Jones, and, although the absolute specificity of the enzymes is not fully established, it explains the occurrence of the different end-products in the different species.

Destruction of Uric Acid.—Man and the higher apes have very little power of destroying uric acid compared with that possessed by other mammals, and hence it is the chief end-product of purine metabolism. Folin (1924) reports that 30–70 per cent. of injected uric acid may be destroyed or transformed by the human organism, the nature of the change being obscure. In the dog, at least, the liver is the principal site of uric acid destruction, as shown by the effect of complete hepatectomy which leads to the appearance of large quantities of uric acid, instead of allantoin, in the urine.

The Uricolytic Index.—This is believed to represent the ability of the animal to destroy uric acid, and is expressed as the percentage ratio of the allantoin nitrogen to the sum of allantoin and uric acid nitrogen excreted.

$$\text{Uricolytic Index} = \frac{\text{Allantoin N} \times 100}{\text{Allantoin N} + \text{Uric acid N}}$$

Among carnivora, the low index of the Dalmatian dog is of note, this animal having a uric acid excretion almost as high as that of man. Onslow has shown that the high index is a dominant Mendelian character, and when Dalmatian dogs are crossed with other dogs, the offspring display the uricolytic efficiency of the majority of the carnivora.

Uricolytic Indices of Higher Animals

Animal.	Order.	Index.
Man	Primates	0 (?)
Chimpanzee	Primates	0 (?)
Monkey	Primates	89
Elephant	Proboscidea	72
Cow	Ungulata	93
Horse	Ungulata	88
Pig	Ungulata	98
Cat	Carnivora	97
Dog	Carnivora	98
Dog, Dalmatian	Carnivora	32
Rat	Rodentia	96
Rabbit	Rodentia	95
Opossum	Marsupialia	79

Purine Metabolism.—On a mixed dietary, the average human renal output of purine, *per diem*, is 0.6–0.9 gm. as uric acid, 30–50 mg. of purine bases (adenine, guanine hypoxanthine and xanthine), and a variable amount of methyl purines, derived from caffeine

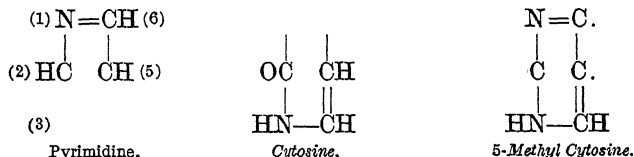
and theobromine. This output corresponds to a uric acid level in the blood of 1.5–3.5 mg. per 100 ml., a value which may be raised tenfold in conditions of renal incompetence, gout, lead-poisoning, and azotæmic nephritis. The intestinal excretion of purine is hard to assess owing to the bacterial destruction of these compounds. At least half the purine output is exogenous, being derived from the nucleoproteins of the dietary, and may be increased considerably by the ingestion of foodstuffs rich in nuclear material, such as sweetbread (pancreas or thymus), liver and kidney.

The human organism has the power of synthesising purines, as is shown by the fact that milk, an adequate food for the growing child, is almost purine-free. The precursors of these purines, which are elaborated to provide nucleoproteins for the cells and tissues, appear to be the amino acids, arginine and histidine, one at least of which is essential for life.

On a purine-free diet of starch and cream, Folin was able to reduce his uric acid output to 0.3 gm. *per diem*, approximately. The experiment was not continued for more than a few days, and this value probably represents the maximal level of the *endogenous* metabolism of purines derived from non-purine sources within the organism. Endogenous uric acid arises from the nucleic acid liberated by the destruction of cell nuclei, especially those of the leucocytes, which are being continually manufactured and broken down, and the erythrocytes, which are denucleated prior to being issued into the general circulation. Any condition of hyperleucæmia or of increased leucocyte destruction, tends to raise the output of endogenous uric acid. The hæmopoietic, or blood-forming apparatus appears to be the chief source of endogenous purine, but, in addition, it is probable that some uric acid is derived from the adenylyl and other nucleotides that participate in carbohydrate metabolism.

PYRIMIDINES

At least four pyrimidine derivatives occur in nucleoproteins, each being referred to a parent pyrimidine not found free in nature.



Like the aminopurines, the aminopyrimidines occur as components of nucleosides, and are liberated as end-products of nucleoprotein digestion. By deamination, *cytosine* (2-oxy-6-amino pyrimidine) is

converted to *uracil* (2 : 6-dioxy pyrimidine) ; and 5-methyl cytosine is converted to *thymine* (2 : 6-dioxy-5-methyl pyrimidine), the change being analogous to the deamination of adenine and guanine.

Thymine was originally obtained as an end-product of the hydrolysis of thymo-nucleic acid, its precursor in the nucleoside is 5-methyl cytosine.

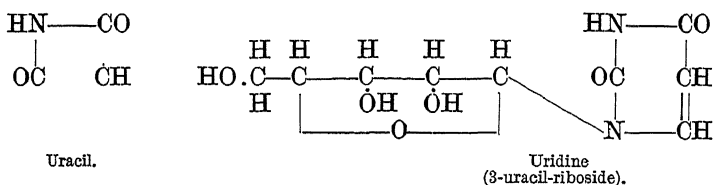
Pyrimidine Derivatives

Pyrimidine nucleosides of cytosine, uracil, thymine, methyl cytosine are obtained as products of nucleic acid hydrolysis. *Vicine*, a D-glucoside of 2, 5-diamino-4, 6-dihydroxy pyrimidine occurs in plants.

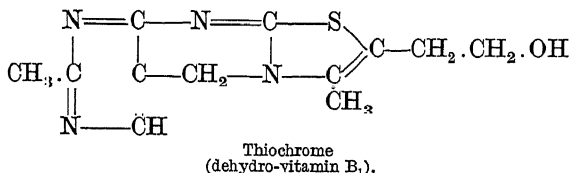
Uridine, uracil nucleoside, is obtained from *uracil nucleotide*, which occurs along with *cytosine nucleotide* in yeast-nucleic acid.

Thymo-nucleic acid contains cytosine and 5-methyl cytosine nucleotides as its pyrimidine components.

In structure, these nucleotides correspond to the purine nucleotides in being phosphoric esters of nitrogenous glycosides.



Pyrimidine Vitamins.—The pyrimidine ring occurs in vitamin B₁, thiamine, and in vitamin B₂, riboflavin (pp. 206, 255), and in the co-enzyme, co-carboxylase, which is a phosphoric ester of thiamine (p. 252). Oxidation of thiamine leads to the formation of *thiochrome*, a pigment found in yeast, and characterised by its fluorescence in ultra-violet light.



Pyrimidine Nucleotides.—Yeast nucleic acid contains two pyrimidine nucleotides, *uracil nucleotide* and *cytosine nucleotide*; thymus nucleic acid contains *cytosine nucleotide* and 5-methyl *cytosine nucleotide*. In structure, these nucleotides correspond to the purine nucleotides, being phosphoric esters of nucleosides, or compounds of pentose and a nitrogenous base. Thus, *uridine* is the nucleoside derived from uracil.

Pyrimidines are very reactive, and readily undergo reversible oxidation, which suggests that they participate in the intense metabolism that is characteristic of nuclear tissues.

Uracil and thymine when fed to dogs are excreted as urea. Cytosine is partly deaminated to uracil, and partly excreted unchanged.

GENERAL REFERENCES

- DRURY, A. N. (1936), "Physiological activity of nucleic acid." *Physiol. Rev.*, **16**, 292.
- FOLIN, O., H. BERGLUND and C. DERICK (1924), "The uric acid problem." *J. Biol. Chem.*, **50**, 561.
- JOHNSON, T. B. (1938), "Pyrimidines, Purines and Nucleic Acids." *Organic Chemistry*. Ed. by H. Gilman. London.
- JONES, W. (1920), "Nucleic Acids." 2nd Ed., Monographs on Biochemistry. London.
- LEVENE, P. A., and L. W. BASS (1931), "Nucleic Acids." American Chemical Monographs. New York.
- ROSE, W. C. (1923), "Purine Metabolism." *Physiol. Rev.*, **3**, 544.
- TIPSON, R. S. (1935), "The Nucleic Acids." *Text-Book of Biochemistry*. Ed. Harrow and Sherwin. Philadelphia.

CHAPTER XXI

NITROGENOUS BASES

THE term *simpler natural bases* has been applied by Barger to describe a type of nitrogen compounds widely distributed in plants and animals. Many are derived from the natural amino acids, and many have a powerful physiological action. As a group, all are water-soluble and precipitated by phosphotungstic acid.

Classification of the Simpler Bases.—(1) *Amines* derived from natural amino acids. Proteinogenous amines.

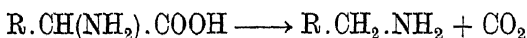
(2) *Betaines*, onium derivatives of methyl amino acids.

(3) *Cholines*, onium derivatives of methyl amino alcohols.

(4) *Guanidines*, derivatives of the base $\text{H}_2\text{N} \cdot \text{C}(\text{NH}) \cdot \text{NH}_2$.

(5) *Miscellaneous bases*.

(1) **Proteinogenous Amines.**—An α -amino acid can give rise to a corresponding amine by decarboxylation, in accordance with the type formula :—



Aminogenic organisms are constant inhabitants of the human intestine, and decarboxylation may be regarded as a by-path in amino acid catabolism. It is probable, however, that most of these autogenous amines are transformed or destroyed before being absorbed, otherwise, a large protein meal would be followed by frequent instead of occasional discomfort.

Sympathomimetic or Pressor Amines.—Amines may be classified in terms of their physiological action into *pressor* and *depressor* amines. The pressors being termed *sympathomimetic* because their action resembles certain effects got by stimulation of the sympathetic nervous system, namely :—

(1) Vascular constriction with accompanying rise in blood pressure.

(2) Increased force of cardiac contraction.

(3) Constriction of the muscular wall and relaxation of the sphincters of the alimentary tract.

(4) Dilatation of the pupil of the eye.

(5) Contraction of the uterus.

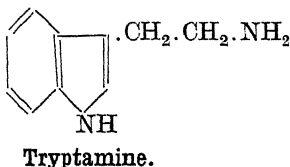
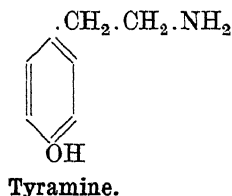
Primary amines are slightly sympathomimetic, the effect being

maximal in *n*-hexylamine, $C_6H_{13}.NH_2$, but the principal pressor bases are the complex amines, tyramine and tryptamine, as well as the hormone, adrenaline.

Tyramine, *p*-hydroxy phenylethylamine, $HO.C_6H_4.CH_2.CH_2.NH_2$, the amine derived from tyrosine, is the chief pressor base found in some extracts of ergot, in putrefied animal tissues, and in "ripe" cheese.

Pharmacologically, tyramine has a weaker, slower, more persistent and less toxic action than adrenaline. Administered orally or intravenously it causes a rise in blood pressure and an increase in the force of the heart beat. It is also an *ecbolic*, causing contraction of the pregnant uterus, but, as some assert, inhibiting the contraction of the non-pregnant uterus. Tyramine gives the characteristic colour tests for tyrosine (p. 149).

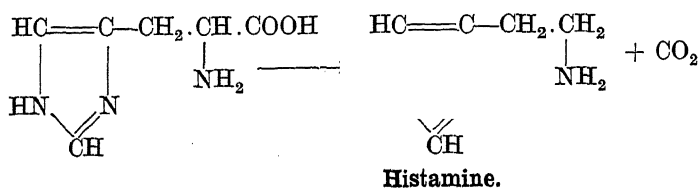
Tryptamine, β -indolyl ethylamine, the amine corresponding to tryptophane, resembles tyramine, but is less powerful. It has been obtained as a product of protein putrefaction, but has not yet been found free in plants.



Depressor Amines.—*Histamine*, β -iminazolyl-ethylamine, the amine derived from histidine, is one of the chief principles of ergot, in which it was discovered by Barger and Dale (1910). It has been obtained also by the putrefaction of histidine and proteins containing histidine. Its chief animal sources are the aqueous or alcoholic extract of lung, liver, spleen, intestinal mucosa, and the posterior lobe of the pituitary gland.

For many years it has been known that simple extracts of animal tissues, when injected intravenously, may cause a profound fall in blood pressure in experimental animals, especially carnivora. This depressor effect is essentially a vaso-dilatation, although cardiac and respiratory phenomena may be involved also. At one time it was believed that the depressor effect was due to choline, but this was disproved when Vincent and others showed that the effect was not inhibited by a previous injection of atropine, the pharmacological antagonist of choline. The histamine is not derived from infective or post-mortem changes in the tissues, as it can be obtained when living lung tissue is taken from an anæsthetised animal, and

straightway frozen in alcohol, which indicates that the amine is immediately available in the living cell, probably as part of the system :—



Detection of Histamine.—(1) *Diazo Reaction* (Pauly).—Freshly prepared diazo reagent is added to a dilute alkaline solution of the amine. A deep red indicates histamine, histidine, and other iminazole derivatives. On acidification, the colour changes to orange. Tyrosine and other *p*-hydroxy compounds give similar colours.

(2) *Bromine Reaction* (Knoop).—Dilute bromine water is added to a neutral or slightly acid solution of the amine. On boiling off the excess of bromine, an orange-brown colour persists if the solution contains histamine or histidine. Hunter has improved the test by removing excess of bromine by shaking the mixture with chloroform previous to heating. (Tryptophane under similar conditions gives a purple colour in the cold, easily destroyed by excess of bromine.) The chief physiological tests for histamine are : (i.) contraction of isolated uterus or intestine of guinea-pig or rat ; (ii.) lowering of blood pressure after injection into cats or dogs ; (iii.) dermatographic response when injected subcutaneously into human subjects ; (iv.) bronchospasm when injected into guinea-pigs.

Physiological Properties of Histamine.—This amine is sharply differentiated from other proteinogenous amines by its powerful depressor effect. Intravenous injection is followed by a quick fall in blood pressure, which is not inhibited by atropine. This is due to a direct action on the capillary wall, causing paralysis, loss of tone, passive dilatation, and increased permeability.

The capillary dilator effect is accompanied by a weaker constrictor action on the arterioles which is insufficient to check general capillary engorgement ; consequently, histamine injection or liberation causes a rapid and often fatal fall in blood pressure. This effect is most marked in carnivora, and may be weak or lacking in anaesthetised rodents, a paradox ascribed to the desensitising effect of many anaesthetics.

Histamine stimulates involuntary muscle, such as that of the

intestinal tract, uterus, bronchioles and arterioles, the response depends on species and on physiological circumstances, and may lead to fatal asphyxia in some animals, notably the young guinea-pig.

Histamine is a powerful secretagogue, and evokes a rapid flow of gastric juice and saliva when injected into the human subject.

The gastric juice thus formed is rich in free hydrochloric acid but poor in enzymes and mucin, a fact attributed to the selective action of histamine in stimulating the parietal oxyntic cells of the gastric mucosa. As secreted in response to food, gastric juice contains a hormone, *gastrin*, which, in turn, evokes the flow of more juice. Gastrin closely resembles histamine, and may be the actual amine.

Putrescine, $\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$, tetramethylene diamine, is derived from the diamino acid ornithine, by decarboxylation.

Cadaverine, $\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$, pentamethylene diamine, is derived from lysine, by decarboxylation. These diamines were formerly regarded as being typical "ptomaines," or poisonous bases produced by the bacterial decomposition of proteins. They are not highly toxic compounds, and are frequent constituents of the urine in the condition of cystinuria (p. 409).

Spermene, $\text{H}_2\text{N} \cdot (\text{CH}_2)_3 \cdot \text{NH} \cdot (\text{CH}_2)_4 \cdot \text{NH} \cdot (\text{CH}_2)_3 \cdot \text{NH}_2$, and *Spermidine*, $\text{H}_2\text{N} \cdot (\text{CH}_2)_3 \cdot \text{NH} \cdot (\text{CH}_2)_4 \cdot \text{NH}_2$, are polyamines isolated and identified by Rosenheim. They occur in extracts of testicle and other tissues.

Spermene is widely distributed in animal tissues, and is manufactured or stored in the prostate gland. Representative values, in mgm. per 100 gm. fresh tissue, are : human prostate, 130 ; human pancreas, 16 ; ox pancreas, 25-30 ; human liver, 10 ; testicle, spleen, kidney, 1-7.

The distribution of these and other amines suggests that decarboxylation can take place in many different tissues.

Amines not Directly Derived from Amino Acids.—Some of the primary amines found in tissues cannot be referred to any known parent amino acid, and must be formed by special synthesis. Among them are :—

Sphingosinol, a primary amine derived from an unsaturated 18-carbon acid. It has the formula $\text{CH}_3 \cdot (\text{CH}_2)_{12} \cdot \text{CH} : \text{CH} \cdot \text{C}_3\text{H}_4(\text{OH})_2 \cdot \text{NH}_2$, and is a constituent of the cerebrosides or glycolipides (p. 168).

Guanidines.—This group includes guanidine, or imino urea, $\text{H}_2\text{N} \cdot \text{C}(:\text{NH}) \cdot \text{NH}_2$, and its derivatives. Guanidine occurs in the seedlings of the vetch after germination, but has not been detected as a free base in the animal body. It is toxic, and on injection

evokes spasms closely resembling those of hypocalcæmic tetany. Guanidine is readily obtained by the oxidation of proteins rich in arginine, and nucleoproteins rich in guanine, and also arises during autolysis of pancreas. The chief biological derivatives are :—

Monomethyl guanidine, $\text{CH}_3.\text{HN}.\text{C}(\text{:NH}).\text{NH}_2$, traces of which occur in mammalian muscle (0.08 per cent.) and in urine. It is asserted that the urinary output is greatly increased after parathyroidectomy.

Glycocyamine, $\text{H}_2\text{N}.\text{C}(\text{:NH}).\text{NH}.\text{CH}_2.\text{COOH}$, guanidine acetic acid, is of interest as a probable precursor of creatine. It has been identified in the organism.

Creatine, $\text{H}_2\text{N}.\text{C}(\text{:NCH}_3).\text{NH}.\text{CH}_2.\text{COOH}$, methylguanidino acetic acid, is the characteristic constituent of all vertebrate muscle. It is discussed subsequently along with its anhydride, creatinine (p. 367).

Arginine, δ -guanidino valine, and canavanine are the only natural amino acids known to contain the guanidine group (p. 138).

Agmatine, δ -guanidino butylamine, $\text{H}_2\text{N}.\text{C}(\text{:NH}).\text{NH}.\text{(CH}_2)_3.\text{CH}_2.\text{NH}_2$, the amine obtained by decarboxylation of arginine, has been detected in ergot.

Galegine, an alkaloid found in Goat's Rue (*Galegia officinalis*), has been shown by Barger and White to be $\text{H}_2\text{N}.\text{C}(\text{:NH}).\text{NH}.\text{CH}_2.\text{CH}(\text{CH}_3)_2$.

Guanidine derivatives have a hypoglycæmic effect, and resemble insulin in causing a fall in the blood sugar level when injected into the circulation. The response, however, is due to a toxic interference with glucose production by the liver and does not represent a true sugar utilisation.

Analytical Reactions of the Guanidines.—(1) *The oxidised nitroferrocyanide test* (Tiegs, 1924; Weber, 1928). The reagent consists of a mixture of one part each of 10 per cent. sodium nitroferrocyanide (nitroprusside), 10 per cent. potassium ferricyanide and 10 per cent. sodium hydroxide, and 9 parts of distilled water. About 1 ml. of the reagent is added to 5 ml. of the solution to be tested, whereupon a colour develops if guanidine or a substituted guanidine be present.

Orange-red.	Red.	Rose-red.	Green.
Guanidine	Methyl guanidine Creatine Creatinine Urea Histidine	Arginine	Cysteine

The test is interfered with by alcohols and by ketones.

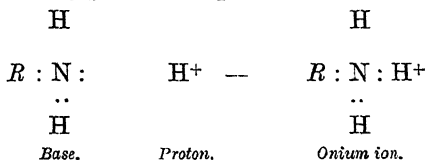
(2) The α -naphthol test (Sakaguchi, 1925) is given by all compounds containing the grouping $\text{H}_2\text{N}.\text{C}(\text{NH}).\text{NH}.\text{R}$, and may be used to detect arginine, glycoeyamine, methyl guanidine, agmatine and other substitute guanidines (p. 148). Free guanidine does not react.

(3) Free guanidine treated with 1 : 2-naphthoquinone-4-sodium sulphonate and alkali yields after about ten minutes a brown solution which turns bright red on addition of nitric acid (Sullivan's test, 1935). Ammonia, methylamine and indole, but none of the natural guanidine derivatives, give similar reactions.

Normal blood has a "guanidine value" ranging from 0.0–0.2 mg. per 100 ml. This may be raised to 0.5 in conditions of arterial hypertension, nitrogen retention, eclampsia, and acute liver injury. It is possible, of course, that these guanidines, whatever they may be, are non-poisonous, and related to creatinine, which, itself, is retained in the blood in some forms of nephritis.

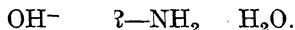
The Onium Compounds

The substances previously considered owe their basicity to the presence of one or more amino groups in which the nitrogen atom is united by *covalencies* to two hydrogen atoms and another group, thus completing an electron octet. In all these compounds, the nitrogen atom possesses a *lone-pair* of unshared electrons, by means of which it is able to form a *semi-polar* union with a proton ion or other positively charged ion. Biological amines are basic in the modern sense of being proton acceptors :



The term "onium" is applied to these cations in which nitrogen is exerting its maximum covalency, the most familiar example being NH_4^+ , the ammonium ion.

Cations of the type $\text{R}.\text{NH}_3^+$, $\text{R}_2.\text{NH}_2^+$ and $\text{R}_3.\text{NH}^+$ are readily attacked by hydroxyl ions, which remove the proton to form water, and release the base,

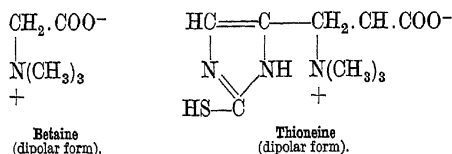


However, when the onium ion is formed from four radicles, and is of the type R_4N^+ a compound results which is stable in aqueous solution and resembles the metallic cations in its power to form

stable salts. The chief representatives of these onium compounds in biochemistry are the betaines, the cholines and amine oxides.

Betaines are cyclic bases found almost exclusively in plants, where they may be storage forms of nitrogen or detoxication products.

One member, ergothionine, or thioneine, has been detected in mammalian blood. It is derived from thiol-histidine, an amino acid, unknown in natural sources.

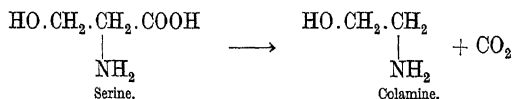


Both betaine and thioneine are neutral in solution owing to their dipolar structure (p. 136).

Refined beet sugar contains traces of betaine as a constant impurity, and thus may be distinguished from cane sugar.

Cholines, or Trimethylamine Bases.—This subgroup includes the highly active and widely distributed base *choline*, together with its derivatives, all of which may be regarded as offspring of the parent, β -amino-ethyl alcohol, or *colamine*, $\text{HO}.\text{CH}_2.\text{CH}_2.\text{NH}_2$.

Colamine is of interest as being a constituent of the cephalins (p. 167), and a possible precursor of the cholines. Its natural origin is unknown, the simplest source being decarboxylation of the hydroxy-amino acid, serine.



Choline, hydroxyethyl - trimethyl - ammonium hydroxide, was originally obtained from bile, and is widely distributed elsewhere in plants and animals as a constituent of lecithin. The choline content of animal mammalian tissue ranges from about 10–30 mg. per 100 gm. fresh material.

Properties of Choline.—Choline and its derivatives constitute, along with histamine, the chief *depressor bases* found in extracts of animal tissues. Intravenous injection of such extracts evokes a fall in the blood pressure of higher animals, the response often being variable and paradoxical. This is ascribed to: (i.) unidentified constituents, (ii.) synergic or reinforcement action among the bases, (iii.) species and other differences among the experimental animals,

(iv.) interference by anæsthetics. Pure choline has a vagomimetic effect, shown by its action in slowing the heart beat and stimulating the movements of the alimentary tract. In addition, it is a secretagogue, stimulating the salivary, sudorific, and lachrymal glands; a myotic, contracting the pupil of the eye; and an ecboic, stimulating the isolated uterus to contract.

Atropine inhibits the vagomimetic effect of choline and unmasks various secondary effects, notably a vaso-constriction. Hence, choline injected subsequently to atropine may exert a pressor action. This paradox is denied by some workers, and is still under investigation.

Acetyl choline attracted attention in 1909, when it was found to have an action similar to choline but 100,000 times more powerful. It is a natural constituent in extracts of ergot, and was subsequently identified in higher animal tissues, when Dale and Dudley (1929) obtained it from fresh spleen. Acetyl choline is the most powerful depressor base known. Intravenous injection causes a sharp and transient fall in blood pressure, due to arteriolar dilatation. If this effect be inhibited by atropine, it is seen that acetyl choline also stimulates ganglion cells in a way similar to nicotine.

Two other properties are of use in analysis. (1) In common with choline, muscarine, and other "parasympathetic" stimulants, acetyl choline increases the tone and rhythm of isolated rabbit intestine suspended in warm saline. (2) Acetyl choline causes a characteristic slow wave of contraction in denervated mammalian voluntary muscle. This action is peculiar to the choline esters.

Acetyl choline is very unstable, and is hydrolysed when warmed with dilute alkalis. By this means it may be distinguished from the relatively more stable choline and histamine. Furthermore, it is rapidly destroyed by disintegrated splenic tissue, and its detection is only possible in extracts prepared under special conditions.

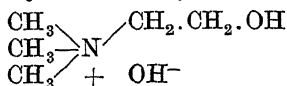
Choline nitrite, or pseudo-muscarine, was at one time thought to be identical with natural muscarine, the highly poisonous alkaloid of the mushroom *Amanita muscaria* (Fly Agaric), and to be the aldehyde corresponding to choline. Both suppositions are wrong. The natural alkaloid is much more toxic than choline nitrite, and its effects are antagonised by atropine, which has little protective effect against the synthetic ester.

Neurine is obtained during the putrefaction of choline or compounds containing choline, such as lecithin. It is a possible contaminant in all crude choline preparations, and a typical example of the class of bases formed during the putrefaction of tissues, and at one time called "ptomaines."

Neurine resembles choline in many physiological properties, but is much more toxic.

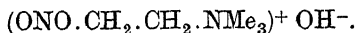
Structure of the Cholines.—Choline is a viscid alkaline liquid, stable in dilute aqueous solutions, but decomposed by heat into trimethylamine, $(\text{CH}_3)_3\text{N}$, and ethylene glycol, $\text{HO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$. It and its relatives are hydroxides of various onium compounds.

- (1) **Choline**, trimethyl-colamine hydroxide,



- (2) **Acetyl choline**, $(\text{CH}_3\text{COO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NMe}_3)^+ \text{OH}^-$.

- (3) **Choline nitrite**, pseudo-muscarine,



- (4) **Neurine**, trimethyl-vinyl-ammonium hydroxide,



Physiological Properties of the Onium Bases.—Onium bases, in general, have three distinct physiological properties : (i.) curari-like paralysis of motor nerve endings in voluntary muscles ; (ii.) parasympathetic or “cholinergic” effect in stimulating tissues supplied by the parasympathetic system ; (iii.) nicotine-like action in paralysing sympathetic nerve ganglia. The intensity and the quality and duration of the particular effect depend both on the structure of the choline derivative and on the previous history of the tissue attacked. Neither the betaines nor trimethylamine oxide have these properties, which must reside in the positively charged onium ions.

The chief significance ascribed to choline in nerve metabolism is its presence as the parent of acetyl choline, the characteristic neurocrine or effector substance of the parasympathetic nervous system. It also influences fat storage (p. 264).

NEURO-HUMORAL MECHANISMS

The existence of a neuro-humoral mechanism or chemical agent intervening between nerve impulse and tissue response has been demonstrated chiefly by the work of Loewi (1921–1930) on the heart, Sherrington on the reflex-arc, and Lewis on the cutaneous capillaries. These chemical agents, or *neurocrines* as they may be termed, resemble the autacoids (Chapter XXIV), but the principal effect is local and more or less limited to the site of their origin. At the same time, two of the best known neurocrines, namely, acetyl choline and adrenaline, function as typical autacoids.

History.—In 1921, Loewi reported that the perfusion fluid from

a frog's heart that had been stopped by vagal stimulation was able to induce typical vagal effects when perfused through another heart, from which he concluded that a "vagal substance" was liberated locally. The work was confirmed by other investigators, who showed that similar substances were liberated in various tissues and glands on stimulation of the parasympathetic nerves, and the term *parasympathin* was introduced to denote the reactant. The term *sympathin* was applied to the corresponding substance liberated locally on stimulation of the sympathetic nerves. Parasympathin is dialysable and acid-stable, but rapidly destroyed by alkalies and by esterases present in blood and most tissues. The destruction by esterases can be prevented by *eserine* (physostigmine), an alkaloid of the Calabar bean.

In all these properties, parasympathin resembles acetyl choline.

In many ways sympathin resembles adrenaline, the secretion of the suprarenal medulla. Both hormones are inactivated when mixed with eosin and exposed to ultra-violet irradiation. Both hormones, when conveyed by the blood stream, evoke acceleration of the heart beat, rise in blood pressure, salivation, and contraction of the nictitating membrane, spleen and pregnant uterus, in the cat. And both have their effects enhanced by previous injections of cocaine.

Adrenaline, however, is able to evoke either contraction or relaxation in unstriated muscle, depending on the nature of the sympathetic innervation, and previous injection of ergotoxin abolishes the vaso-constrictor effect, without inhibiting the vasodilator effect. The properties of sympathin differ somewhat according to the source, and Cannon concludes that two distinct hormones exist: sympathin E, which is obtained from regions in which sympathetic stimulation causes contraction; and sympathin I, which is obtained from muscular tissue which is inhibited by sympathetic stimulation. Following a suggestion of Dale, nerve fibres are now classified as *cholinergic* and *adrenergic*, according to the type of neurocrine liberated. The hormones are secretions of the nerve terminals, themselves, and not products of the excited tissue (Parker, 1932).

The manner in which a neurocrine operates is uncertain. Presumably it is set free by an activation or change in valency electrons in the parent substance as the result of the arrival of a nerve impulse.

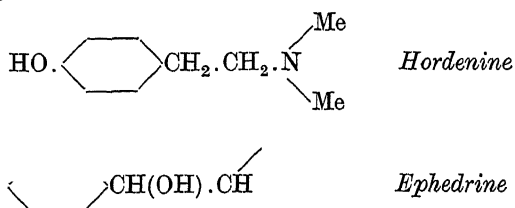
The liberated neurocrine then reacts with some tissue constituent and starts the series of chemical changes involved in muscle contraction or gland secretion.

Neurocrines are characterised by (a) lability, and (b) sensitivity to specific antagonists. Lability is shown by the rapid disappear-

ance of the free neurocrine, otherwise its effect would persist long after the cessation of the evoking stimulus.

Definition and Classification.—A neurocrine, or neuro-humoral factor, is a specific reactant liberated at a nerve ending as the result of nerve stimulation.

(1) *Sympathins*, the sympathomimetic substances liberated in the heart, intestine, and elsewhere as the result of adrenergic stimulation; one of them has been identified with adrenaline. Natural compounds with a sympathin effect are: vaso-pressin (p. 349), tyramine, *l*-adrenaline, hordenine (an alkaloid from barley) and *l*-ephedrine (an alkaloid from the Chinese plant, *Ephedra equisetina*).



(2) *Parasympathin*, the vagomimetic substance liberated in the heart and elsewhere as the result of stimulation of the vagus or an other cholinergic nerve supply. It is a choline ester, and has been identified with acetyl choline. The parasympathin effect is antagonised by atropine, an alkaloid from belladonna, and is reinforced by eserine. Pilocarpine, the alkaloid from jaborandi, is a typical parasympathin, and its inhibitory effect on the heart is antagonised by atropine.

(3) *Synapsin*, the synaptic substance liberated at the synapses of the reflex-arc, determines the phenomena of "recruitment" and "after-discharge" displayed by the nervous system (Sherrington).

(4) *Vaso-dilatin*, or "H-substance" (Lewis), is liberated locally as a result of injury to the skin, such as scalding, burning, irradiation and freezing. By direct action it is responsible for capillary dilation and wheal formation, and through an axon-reflex it evokes a red flush, or "flare," due to arteriolar dilation in the surrounding skin area. H-substance has been identified with histamine.

Summary.—The principal vaso-pressor factors obtained from natural sources are: tyramine, tryptamine, ephedrine, adrenaline and pituitary vaso-pressin.

The principal depressor factors are: histamine, choline and its esters, adenosine, adenylic acid, and pancreatic kallikrein.

CREATINE AND CREATININE

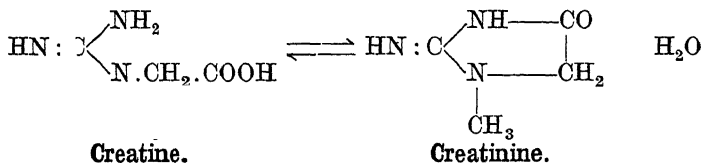
Creatine, or methylguanidino acetic acid, was discovered in meat extract, and named by Chevreul in 1835. Since then it has been shown to be a constant constituent of vertebrate muscle.

Average values in mg. per 100 gm. fresh tissue are : man, 390 ; dog, 370 ; cat, 450 ; horse, 400 ; ox, 430 ; rabbit, 520 ; pigeon, 447 ; frog, 270–450 ; cod, 353 ; skate, 280. The total muscle creatine of a 70 kg. man ranges from 112–140 gm. Creatine is maximal in voluntary muscle, and minimal in involuntary muscle. Traces occur in other organs and tissues, usually one-twentieth or less of that found in the muscles of the same animal. Creatine appears to be absent from the muscles and other tissues of invertebrates.

Properties and Reactions of Creatine.—Commercial creatine is now available as a by-product of the manufacture of meat extract, from which it can be purified by recrystallisation from hot water. It is obtained in hard, colourless prisms, soluble in water to the extent of 1 : 74 at 18° C., and freely soluble at 100° C. Unlike the natural bases, creatine is not precipitated by phosphotungstic acid.

Structure.—The constitution of creatine is uncertain. Its conventional synthesis and many of its reactions indicate that it is a guanidine derivative of sarcosine. The solute, however, is slightly acid, and has none of the basic properties associated with the guanidines or the amino group. Unlike the amino acids, it does not react readily with nitrous acid or with formaldehyde, and on boiling with weak alkalis it is hydrolysed to sarcosine and urea.

Dehydration.—On boiling with dilute acids, creatine may be converted completely into its anhydride *creatinine*. A similar change takes place slowly in aqueous solution, an equilibrium being reached, the constant of which depends on the temperature.



This conversion is the basis of the methods for estimating creatine.

Significance of Creatine.—Creatine is an essential reactant in the contraction process in vertebrate muscle. In the resting state, it is mostly combined with phosphoric acid as a non-diffusible *phosphagen*. Activity, fatigue, injury or death of the muscle causes the resolution of phosphagen into creatine and phosphoric acid, 150 calories being liberated for each gram of acid released. During

the recovery stage, phosphagen is resynthesised, the energy for this being obtained by the breakdown of the adenylyl pyrophosphate in the muscle. Creatine thus serves as a phosphoric acid carrier in metabolism.

Creatine phosphagen is only found in the muscles of vertebrates, its function among the invertebrates being fulfilled by arginine phosphagen (p. 313).

Creatinuria.—Creatine is a normal constituent of the urine of young vertebrates, and is almost always present in the urine of infants and children. It disappears from the urine of the adult man when the muscular system has reached the average percentage of total body weight. It persists intermittently in the urine of women, and is constant in the urine of many lower animals, including cattle, sheep, and the fox. Creatine is an important constituent of the urine of birds, being much in excess of the creatinine present. Creatinuria occurs even if the dietary be creatine-free, showing that it is a product of animal metabolism.

Types of Creatinuria.—(1) *Creatinuria of growth*, as found in children of both sexes up to puberty. It is ascribed to overproduction at an age when the storage capacity of the muscles is low, and is an overflow creatinuria.

(2) *Metabolic creatinuria* may follow nutritional disturbances due to excess of protein or deficiency of carbohydrate in the diet. It appears during pregnancy, lactation, hyperthyroidism, and diabetes.

(3) *Starvation creatinuria* is caused by autolysis of muscle during febrile conditions and starvation.

Creatinuria is characteristic of the last months of pregnancy, and in the few days immediately preceding parturition it may be as high as 170 mg. *per diem*.

After parturition, the output rises to a maximum between the fourth and sixth day, and subsides to the normal value usually by the end of the month.

(4) *Creatinuria of low storage* is seen in muscular dystrophies and atrophies, where the administration of a small quantity of creatine leads to its rapid excretion in the urine.

CREATININE

Creatinine, or methylglycocyamidine, the cyclic anhydride of creatine, was discovered in human urine by Heintz and by Pettenkofer, independently, in 1844, as a constituent precipitated by zinc sulphate or chloride.

Later investigation has shown creatinine to be a characteristic solute in all mammalian urine, ranking next to urea in quantitative importance.

Average percentages are : man, 0.06–0.2 : goat, 0.038 : ox, 0.11 ; sheep, 0.14 ; horse, 0.19. Creatinine also occurs in mammalian blood in concentrations of about 0.1–0.5 mg. per 100 ml. It is easily excreted by the kidney, being a non-threshold solute, and hence creatinine retention only occurs in severe renal dysfunction, when blood values greater than 4 mg. per 100 ml. may be reached. The creatinine content of tissues is about the same or less than that of the blood. Traces occur in many vegetable materials : cereals, potatoes, bran, straw, and soils.

Reactions of Creatinine.—(1) *Hydrolysis*.—On prolonged boiling in neutral or alkaline solution creatinine is in part slowly hydrolysed to creatine and in part resolved into urea and sarcosine. The transformation to creatine is complete if the creatine be removed from time to time by cooling and concentrating the solution.

(2) *Precipitation*.—Creatinine is more basic than creatine, and, unlike it, is precipitated by most of the alkaloidal reagents including phosphomolybdic and phosphotungstic acid. Warmed with Fehling's reagent in presence of sodium carbonate a slow-forming precipitate of creatinine cuprous oxide appears, and may mask a positive carbohydrate reaction in diabetic urine.

(3) *Analytical Reactions*.—These are considered in connection with the detection of creatinine in urine (p. 399).

Creatinine Excretion.—By the use of a colorimetric method, Folin, in 1904, showed that when the diet is free from creatine or creatinine the output of creatinine in the urine is "a constant quantity, different for different individuals, but wholly independent of quantitative change in the total amount of nitrogen eliminated."

Subject.	High Protein Intake, 19 gm. N. per diem.		Low Protein Intake, 1 gm. N. per diem.	
	Total Urinary N in gm.	Creatinine in gm. per diem.	Total Urinary N in gm.	Creatinine in gm. per diem.
1	12.2	1.58	4.1	1.48
	14.5	1.66	4.2	1.60
	16.1	1.49	3.8	1.57
2	11.5	1.63	5.3	1.58
	12.0	1.57	4.8	1.61
	14.7	1.58	3.6	1.60
3	14.6	1.05	2.7	1.17
	15.8	1.16	3.7	1.21
	14.4	1.12	2.8	1.13

Data such as these indicate that the creatinine output is independent of a tenfold increase or decrease in the nitrogen of the diet. Minor variations in output occur during long experimental periods, and are ascribed to variations in the body weight of the subject, especially as regards muscular tissue.

Significance of Creatinine.—Creatinine is a typical waste-product of the animal, and when administered under conditions that ensure full absorption it is rapidly though not quantitatively excreted. The close chemical relationship between creatine and creatinine has long suggested that creatinine is the form in which surplus creatine is excreted. Folin upset this assumption in 1906 when he showed that administration of creatine over three-day periods had no influence on the creatinine output, and he concluded that the metabolism of the two compounds was biologically independent. This paradox was only explained in later years, when it was demonstrated that creatine conversion into creatinine only takes place when a saturation value has been reached in the tissues.

Biological Transformation of Creatine into Creatinine. The results of prolonged feeding experiments with creatine on the dog (Benedict and Osterberg, 1923) and the human subject (Chanutin 1926) show that four stages may be recognised :—

(1) During the first week no change is detectable in the normal output of creatine and creatinine. This is the type of result obtained by Folin in 1906, and if the experiments were carried no further they would serve simply to confirm his work.

(2) During the second week and subsequent weeks the administered creatine can be detected in the urine, showing that the organism is becoming saturated, and an overflow creatinuria is appearing.

(3) As the overflow creatinuria develops, an increase in the creatinine output begins, and continues to rise to a maximal value.

(4) On stopping the administration of creatine, the overflow creatinuria stops at once, but the excess creatinine output falls as slowly and regularly as it originally rose.

Experiments of this type show that urinary creatinine arises from creatine.

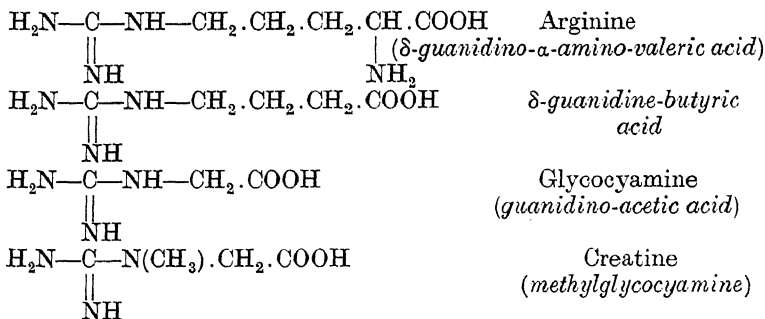
The Creatine-creatinine Metabolism of the Organism.—The creatine content of the human organism is maintained partly by the consumption of animal foodstuffs containing preformed creatine and partly by the synthesis of creatine from unidentified precursors. In the absence of preformed creatine, the animal is able to meet all its requirements synthetically, as shown by the herbivora and other vegetarians. Human blood contains 2–9 mg. of creatine per 100 ml. Creatine, like other valuable metabolites, has a high renal threshold value. It is filtered from the plasma by the glomeruli, and

reabsorbed during its passage along the tubule, probably by being phosphorylated by the renal cells, which thus produce a favourable diffusion gradient. When the creatine content of the blood exceeds 7 mg. per 100 ml. it exceeds the renal threshold level. Creatine enters the tubule more rapidly than it can be reabsorbed, and the excess escapes in the urine.

Creatinine, on the other hand, is a waste product, and of no use to the organism. It has a minimal renal threshold value, and though the blood creatinine content is assessed at 0.1–0.5 mg. per 100 ml., these figures probably include other compounds. Creatinine is filtered from the plasma by the glomeruli, but does not undergo reabsorption during its passage along the tubules. Its excretion rate is the most constant of any of the urinary solutes, being dependent primarily on the blood flow through the kidney. The creatinine of the plasma is one of the first solutes to increase in conditions of renal dysfunction involving nitrogen retention, and Myers claims that a blood creatinine value above 1.5 mg. per 100 ml. is evidence of renal trauma.

In the resting muscle, at least 95 per cent. of the creatine is in the bound form of creatine phosphagen, and is maintained at this level by the plasma creatine, which is in equilibrium with the free creatine of the muscle. Creatine is continually being synthesised in accordance with the phosphagen requirements of the muscular and possibly other systems, the surplus being excreted as creatinine.

The Precursors of Creatine.—Creatine has never been obtained as a product of protein hydrolysis, and must be derived from some parent compound. Among these alleged pro-creatines are: guanidine (Kutscher, 1904) choline (Koch, 1904), arginine (Czernecki, 1905; Thompson, 1918), purines (Forsbach, 1908), cystine (Harding and Young, 1919), and glycine (Brand, 1929). Of these, the most likely is arginine, the protein constituent long known to contain the guanidine nucleus. The conventional biochemical relationship is indicated as follows:—



Conversion of Arginine into Creatine.—This degradation involves the accepted biochemical operations of α -deamination, β -oxidation, with shortening of the carbon chain, and selective methylation. The objections to this change, however, are many.

(i.) Arginine probably is not one of the essential amino acids in nutrition, whereas creatine is an indispensable constituent of mammalian muscle.

(ii.) Arginine is attacked by the specific enzyme, arginase, the presence of which in liver suggests that there can be but little surplus arginine available for creatine synthesis.

Glycocyamine has been detected in animal tissues, but workers disagree as to whether its administration causes a significant increase in muscle creatine or urinary creatinine.

The following observations support the theory that arginine is a precursor of creatine in animal metabolism :—

(i.) When quantities of arginine, up to 1.6 gm. per kg. body weight *per diem*, are injected into dogs, 50–96 per cent. of the amino nitrogen is soon excreted as urea in the urine. If, however, administration by injection or feeding be continued over a long period, there is a gradual increase in the creatinine output, corresponding in some experiments to a conversion of 8–25 per cent. of the guanidine nucleus of the arginine (Thompson).

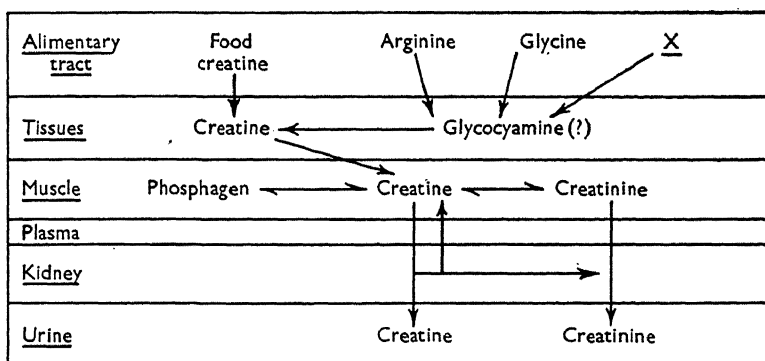
It must be noted, however, that the formation of creatinine from arginine does not necessarily prove that creatine has been formed as an intermediate metabolite, and neither Hyde and Rose (1930) nor Chanutin (1930) could detect any change in the creatine or creatinine output in man and in the rat, respectively, after prolonged administration of arginine.

(ii.) By perfusing the isolated heart with salines containing arginine, Fisher and Wilhelmi (1938) obtained an increase in creatine closely corresponding to the amount of arginine that disappeared. They ascribe the inconclusive results obtained by feeding or injection to the great capacity possessed by hepatic tissue for destroying arginine.

(iii.) In the muscle of invertebrates, arginine phosphate occurs as a phosphagen instead of creatine phosphate, which suggests a biological substitution in the evolutionary scale.

Site of Creatine Formation.—Harding assesses the *total creatine coefficient*, or daily output of creatine and creatinine (expressed as creatine) as approximately 23 mg. per kg. body weight *per diem*, and claims that production is not dependent on the muscular system but is a function of the total body weight. The immediate precursor he regards as glycocyamine, which may come from the degradation of arginine, or from glycine, through the intermediate

stage of hydantoic acid. The *creatinine coefficient* has been assessed by Folin at 18–32 mg. per kg. body-weight *per diem*, the average being 24 for men, and 16 for women. Removal of the liver in dogs does not affect the level of blood creatinine, showing that its formation must be extra-hepatic. According to Benedict and Behre, creatinine is absent from the blood of many animals, showing that the creatinine of urine must arise in the kidney. While creatine is an obvious precursor of creatinine, it is possible that both these compounds may arise from a common ancestor, the fate of which is determined by the creatine requirements of the organism. Free creatine accumulates in muscle during the contraction process, and some of it may be dehydrated to creatinine. Reabsorbed creatine in the renal tubules is partly dehydrated to creatinine, and may account for at least two-thirds of the total urinary creatinine.



Creatine-Creatinine Metabolism

Miscellaneous Bases

Ammonia.—Although ammonia is formed by the deamination of amino acids and purines in many parts of the body, the free ion is found only in urine as a normal constituent. Traces of ammonia appear in saliva and in intestinal contents as a result of bacterial changes.

Circulating blood, as Conway has shown, has an ammonia value of zero, or below analytical level. After shedding, ammonia arises from three sources : (i.) α -ammonia, which appears almost immediately, and has a nitrogen value of 40% per 100 ml. It comes from the action of plasma deaminase on plasma adenosine, which, in the circulation, is protected by plasma CO_2 .

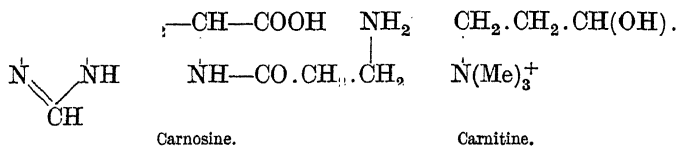
(ii.) β -Ammonia (1 mg. per 100 ml., in rabbit's blood), arises later, from deamination of adenylyl triphosphate in the red cells.

(iii.) γ -Ammonia (0.35 mg. per 100 ml. in the rabbit) arises finally, from vegetable adenylic acid.

Carnosine, β -alanyl histidine, forms about 0.2–0.3 per cent. of mammalian muscle. It is water-soluble, dextro-rotatory, and like histidine, gives a diazo reaction, and acts as a pressor base. Its significance is unknown but it is of interest in that it is a natural derivative of a β -amino acid.

Anserine, or methyl carnosine, occurs to the extent of about 0.1 per cent. in the muscles of the goose and other birds, where it appears to replace carnosine.

Carnitine is an onium base present in mammalian muscle in amounts from 0.02 per cent. (rabbit) to 0.1 per cent. (ox.)



Trimethylamine, $(\text{CH}_3)_3\text{N}$, occurs in the sexual tissues of many plants and animals, in association with the sex hormones.

Trimethylamine oxide, $(\text{CH}_3)_3\text{N}^+\text{O}^-$, an onium salt found in marine but never in fresh-water fishes. Rich sources are cephalopods and crustaceans, the muscle of the lobster containing about 0.3 per cent. The oxide is soluble, non-toxic and almost neutral, and is an important excretory form of nitrogen. Among the elasmobranchs it serves in the maintenance of fluid equilibrium, and is responsible for 20–25 per cent. of the total osmotic pressure of the blood. It is rapidly decomposed by post-mortem autolytic and bacterial changes, and the liberated trimethylamine, $(\text{CH}_3)_3\text{N}$, characterises the odour and taste of stale marine fish.

Tetramethyl ammonium hydroxide, $(\text{CH}_3)_4\text{N}^+\text{OH}^-$, is the toxin in the stings of jellyfish, and displays marked curare-like properties.

Nicotinic amide, $\text{C}_5\text{H}_4\text{N.CO.NH}_2$, is a constituent of the co-enzymes, co-dehydrogenase I and II, and as such acts as a hydrogen carrier in tissue respiration. The amide is derived from nicotinic acid, or carboxy pyridine, and is combined with ribose phosphate and adenosine in nucleotide structure in the co-dehydrogenases. Its hydrogen-carrying power is due to the reducibility of the onium nitrogen in the pyridine ring (p. 258).

Benzedrine, $(\text{C}_6\text{H}_5)\text{CH}_2.\text{CH}(\text{NH}_2).\text{CH}_3$, β -phenyl isopropylamine, a synthetic base, was introduced into therapeutics in 1935, by Prinzmetal and Bloomberg. It is an adrenergic type of base, resembling adrenaline and ephedrine, but displaying a marked psycho-kinetic effect. For this reason, it has been employed

in 10–20 mg. doses in the empirical treatment of mental disorders. Quastel and Wheatley (1933) have shown that aromatic amines, such as β -phenyl ethylamine, tyramine and indole, inhibit strongly the oxidation of glucose, lactate and pyruvate by brain tissue, so it is probable that the effect of benzedrine represents a perversion rather than a stimulation of cerebral metabolism.

PLANT ALKALOIDS

Characteristic of many species of plants are the presence of complex derivatives of pyridine, quinoline and other heterocyclic nuclei. Because of their basic properties, these substances are termed alkaloids. In complexity of structure and obscurity of function, the plant alkaloids excel the known animal bases, and they are the subject of specialised study because of the powerful and often unique physiological properties which many of them possess.

GENERAL REFERENCES

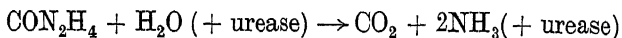
- ALLES, G. A. (1934), "Physiological significance of choline derivatives." *Physiol. Rev.*, **14**, 276.
- BALDWIN, E. (1937), "Comparative Biochemistry." Cambridge.
- BARGER, G. (1914), "Simpler Natural Bases." Monographs on Biochemistry. London.
- BARGER, G. (1931), "Ergot and Ergotism." London.
- BEST, C. H., and E. W. McHENRY (1931), "Histamine." *Physiol. Rev.*, **11**, 371.
- CANNON, W. B. (1933), "Chemical mediators of autonomic nerve impulses." *Science*, **78**, 43.
- DALE, H. H. (1929), "Transmission of the nerve impulse." Croonian Lectures, *Lancet*, **216**, 1285.
- HENRY, T. A. (1939), "Plant Alkaloids." 3rd Ed., London.
- HUNTER, A. (1928), "Creatine and Creatinine." Monographs on Biochemistry, London.
- MAY, P. and G. M. DYSON (1939), "Chemistry of Synthetic Drugs." 4th Ed., London.
- PARKER, G. (1932), "Humoral Agents in Nervous Activity." Cambridge.
- SHIVER, H. E. (1928), "Physico-chemistry of creatine and creatinine." *Chem. Rev.*, **6**, 419.
- SOLLMANN, L. (1922), "Pharmacology of the autonomic system." *Physiol. Rev.*, **2**, 479.
- STEPHENSON, M. (1938), "Bacterial Metabolism." 2nd Ed., Monographs on Biochemistry, London.

CHAPTER XXII

UREA

Urea, or "Carbamide," CON_2H_4 , is the principal form in which nitrogen leaves the higher organism, and is the chief nitrogenous constituent of the urine of vertebrates other than birds and snakes, where it is replaced by uric acid. Urea is present also in lower animals, in fungi, and in some higher plants. It is one of the simplest and most widely distributed of the biological nitrogen compounds, and is a frequent end-product in the final degradation of amino acids.

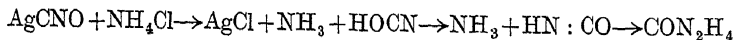
History.—The ammoniacal fermentation of urine, which takes place spontaneously when urine is exposed for some days to the air, depends on the conversion of urea into ammonium carbonate by the urease-forming micro-organisms that abound in the environment.



This change had been known before the time of Pliny, and, in 1773, Rouelle isolated urea by alcoholic extraction of the residue got by the careful evaporation of fresh urine. In 1798, Fourcroy and Vauquelin obtained urea nitrate, $\text{CON}_2\text{H}_4 \cdot \text{HNO}_3$, as a crystalline precipitate on addition of excess of concentrated nitric acid to the urine of man and other animals, and demonstrated that "this special material of the urine, which we now call urea [Urée], . . . gives rise to the carbonate of ammonia which replaces it in the putrefaction of urine."

Wöhler, in 1828, announced his famous synthesis of urea from inorganic materials, and, in so doing, bridged the abyss between organic and mineral chemistry.

The synthesis was the result of an attempt to prepare ammonium cyanate by the action of ammonium chloride on silver cyanate, or ammonium hydroxide on lead cyanate. In each reaction a crystalline product was obtained which was identical with urea, previously found only in urine. The mechanism of the synthesis has been explained by Werner. Cyanic acid, HOCN , readily changes into its isomer, *iso*- or keto-cyanic acid, $\text{HN}:\text{CO}$, which unites with ammonia directly to form urea.



Distribution of Urea in Animals.—Urea is present in the blood and tissue fluids of all vertebrates and in the urine of all mammals.

It also occurs in many invertebrates, including Echinoderms, Crustaceans, Molluscs, and Nematodes.

Elasmobranchs (Dog fish, Skate, Shark) are noteworthy for having an exceptionally high concentration of blood urea.

*Urea Concentrations in Blood of Mammals and Non-mammals.
Expressed as Urea N in mg. per 100 ml., and also as Urea N
percentage of total non-Protein N.*

Animal.	Urea N mg. per cent.	Urea N. per cent. n.p. N.
Man :		
Average range of 211	5.0-11.5	35.2
Plasma, average	12.4	50.2
Serum	11.0-26.0	—
Monkey	38.0	63.3
Dog	11.1-16.7	37.9
Cat	34	56.6
Rabbit	13	41.9
Ox	14	58.3
Horse	7.9-15	—
Pig	14	43.7
Rat	22	57.9
Duck	7.0	29.1
Chicken	8.0	24.4
Mackerel	10.0	11.5
Eel	9.0	18.0
Frog	7.5	—
Dog-fish	800.0	80.5
Sand-shark	868.0	80.0

This method of expressing values in terms of nitrogen concentration is used chiefly in clinical investigation. The urea value is approximately twice that of the urea nitrogen, since 60 gm. urea equal 28 gm. urea N. With few exceptions, the recorded values for mammalian blood urea N average about 12 mg. per 100 ml. blood. In birds and fishes the value is about half this, the undetermined N being remarkably high.

As a solute, urea is universally and uniformly distributed throughout the organism, the concentrations being approximately the same as in the blood. Outstanding exceptions are the adipose tissue, which has a low urea value corresponding to its low content of water, and the renal tissue, which has a high value, owing to the presence of urine.

Excretion of Urea.—Urea leaves the animal in three ways: the kidney, the skin, and the intestine. Of these, the first is by far the most important, but in conditions of renal inefficiency the cutaneous excretion of urea may become considerably greater than its normal human value of 0.1 gm. *per diem*. Little is known about the intestinal secretion of urea; in health it is probably negligible.

The urinary output of urea depends primarily on the protein content of the dietary. On a European standard of 80–100 gm. protein *per diem*, the daily output is about 28 gm. urea, representing 80–89 per cent. of the total urinary nitrogen.

According to Van Slyke, when the rate of secretion of urine exceeds an "augmentation limit" of 2 ml. per minute, its urea content is directly proportional to the urea in a given volume of blood passing through the kidney in unit time, the maximal value of which is about 75 ml. of blood per minute, which affords the "maximum clearance" of urea from the blood.

The urea content of mammalian urine is greatest in Carnivora, average percentage values being dependent on protein intake. *E.g.*, Man, America, 2.6; France, 2.2; Germany, 1.9; tiger, 6.9; cat, 2.2; rat, 4.5; cow, 0.9–2.6; horse, 1.5–2.6; rabbit, 0.2–0.4.

In human secretions other than urine, urea is present in the following percentages: cerebro-spinal fluid, 0.2–0.4; saliva, 0.09–0.4; milk, 0.08–0.16; bile, 0.18–0.4.

Among plants, urea is found in many fungi and moulds in fairly high concentration. Green plants contain traces, probably as unstable ureides, or carbamido acids, such as citrulline.

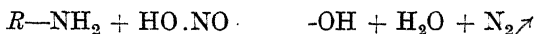
Properties of Urea.—Urea is a white, crystalline solid, with a faint, salty taste. It separates from aqueous solutions in long prismatic crystals that melt at 132.6° C. (corr.), and above this temperature dissociate into cyanic acid and ammonia. Urea is very soluble in water, glycerol, and methyl alcohol, and to a less extent in hot ethyl alcohol and in acetone. It is insoluble in ether, chloroform, and benzene.

Solubility in water, expressed in gm. urea per 100 gm. of solvent, is: 55.9 at 0° C.; 66.0 at 10° C.; 79.0 at 20° C.; 93.0 at 30° C.; 106.0 at 40° C.; 120.0 at 50° C.; 145.0 at 70° C. (Speyers, 1902).

Urea is neutral, but can react as a monobasic amide and form crystalline salts with strong acids. These, especially the nitrate and the oxalate, are insoluble in excess of acid, and may be used for the separation and identification of urea.

In alkaline solution, urea forms insoluble compounds with salts of the heavy metals, such as AgNO_3 and HgCl_2 .

Nitrous acid specifically attacks the group —NH_2 and replaces it by —OH , the nitrogen escaping as a gas :—



This reaction is employed by Van Slyke in a well-known method for estimating amino acids. The response of urea is altogether atypical. When nitrous acid, freshly prepared by the addition of a nitrite to a slight excess of acetic acid, is brought into contact with urea the mixture may remain unchanged for hours. If, however, a strong acid (hydrochloric or nitric) be added, a brisk reaction begins, and is completed rapidly, even in dilute solution, but the volume of nitrogen gas evolved is always less than the amount of urea decomposed. This suggests that the amide groups of urea are masked in neutral or faintly acid solution, and only partially exposed by the action of strong acids. Aldehydes react with urea only if the solution is sufficiently acid or alkaline to unmask the —NH_2 (p. 142).

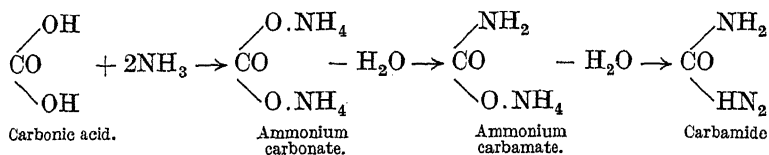
Preparation of Urea from Urine.—(a) *As Urea Nitrate.*—When normal human urine is treated with an equal volume of concentrated nitric acid crystals of urea nitrate may begin to separate out within an hour. Many specimens of urine, however, yield no precipitate even after twenty-four hours. This is not always due, as might be expected, to a lower concentration of urea in the urine, but depends on the presence of a colloid that inhibits precipitation. An aqueous solution of urea of the same concentration as that in urine (2 per cent.) gives a dense precipitate of urea nitrate within five minutes after addition of an equal volume of nitric acid, if the liquid be kept cool. Consequently, in preparing urea nitrate from its natural source, the urine must be concentrated to about a quarter of its original volume by evaporation on a water bath. An equal volume of pure nitric acid is added to the cold concentrate, and after twenty minutes the crystalline precipitate of urea nitrate is filtered off, neutralised with barium carbonate suspension, evaporated to dryness on a water bath, and the urea extracted by hot alcohol.

(b) *As Free Urea.*—The urine is evaporated to a paste on a water bath, and then extracted repeatedly with hot acetone. Urea thus obtained is very crude, and must be decolorised by being boiled with charcoal before it is purified by recrystallisation from acetone.

The Constitution of Urea.—Although urea is a simple compound built up of eight atoms, at least six different formulæ have been proposed to explain its constitution and reactions.

(1) *The molecular formula*, CON_2H_4 , follows from the fact that urea has a molecular weight of 60, as shown by the osmotic pressure and freezing point of the solution.

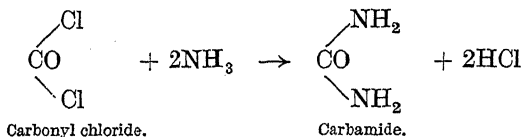
(2) *The carbamide formula*, $\text{CO}(\text{NH}_2)_2$ (Dumas, 1830). According to this early formula, urea is the diamide of carbonic acid and can be prepared by the general reaction for the preparation of amides, namely, the dehydration of the corresponding ammonium salt.



Ammonium carbamate is obtained by heating ammonium carbonate, and the further dehydration to carbamide seems obvious.

This synthesis was first accomplished by Basarov in 1868, who obtained a 3 per cent. yield of urea after heating ammonium carbamate for four hours under pressure at 140°C .

The synthesis of urea from the action of ammonia on carbonyl chloride appears to proceed in accordance with the carbamide formula :—



The carbamide formula for urea is widely accepted, and serves to explain, superficially at least, many of the reactions of the compound.

(3) *The amidine or iso-carbamide formula*, $\text{HO.C}(\text{NH})\text{.NH}_2$ (Butlerov, 1868).—Urea is a neutral solute, and has not the alkalinity implied by the presence of two amide groups. With strong acids only monobasic salts are formed, for example, urea nitrate, $\text{CON}_2\text{H}_4\text{.HNO}_3$. The difficulty with which urea is obtained by the action of heat on ammonium carbamate is in striking contrast with the ease with which amides are prepared from the corresponding ammonium salts.

These and other considerations led Butlerov to suggest that urea existed in solution as an isomer of carbamide, and he appears to have been the first to suggest the formula, subsequently adopted by Wanklyn and Gamgee (1868), according to which urea is an unsymmetrical structure possessing only one amido group.

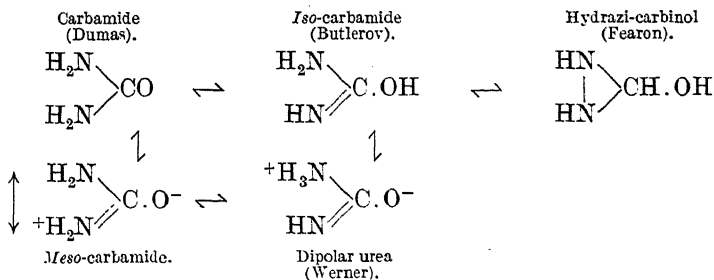
The dipolar formula, $-\text{O.C}(\text{NH})\text{.NH}_3^+$ (Werner, 1912–1923).—To explain the absence of typical amide properties, and to account for the readiness with which urea is formed from, and dissociated into, ammonia and cyanic acid, Werner, in 1912, proposed a formula

for urea in which the amide and hydroxy groups were held by mutual attraction in neutral solution and released by the presence of strong acids or alkalis. When the formula was first proposed the existence of dipolar or zwitter-ions had not been accepted by chemists, and Werner represented the urea as a cyclic structure. This has been criticised on the grounds that one of the nitrogen atoms is depicted as pentavalent, which was not Werner's intention. The dipolar form reverts to the amidine form under the influence of acids, alkalis or heat, when in solution.

The dipolar form has been shown by Clow (1936) to explain the properties of crystalline urea, although there is an uncertainty as to whether it is the amido group or the imino group which carries the positive charge (Sidgwick, 1936). That is to say, crystalline urea may be $-O.C(NH).NH_3^+$ or $-O.C(NH_2)NH_3^+$.

(5) *The Cyclic Formula.*—Urea is a neutral solute, and has neither the base-neutralising power implied by the presence of an $-NH_3^+$ group, nor the acid-neutralising power implied by $-NH_2$. Unlike the amino acids, which are typical dipolar compounds, free urea does not immediately react with formaldehyde nor with nitrous acid, but only does so when in strongly acid solution. This suggests that the amide groups are masked by mutual combination, possibly as a *cyclic amidine* or *hydrazine* ring that requires to be unlocked by a strong acid or alkali before the groups can react.

(6) *The resonance formula, meso-carbamide.*—Measurements of the physical properties of urea, such as the Raman spectrum, the dipole-moment and the parachor, yield data, some of which are not yet fully interpreted, but which indicate that urea in solution is a mixture of isomers in a resonance equilibrium that is sufficiently stable to conceal the chemical properties of the amido group. Urea is thus represented as a hybrid structure, *meso-carbamide*, that differs from the Werner dipolar form in having the displaced H atom shared between the amido and the imino groups.



The Five Structural Formulæ for Urea

REACTIONS OF UREA (Werner, 1923)

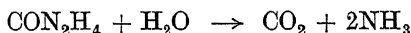
(1) **Decomposition by Heat.**—Heated above its melting point (132°C.) urea readily dissociates into cyanic acid and ammonia :—



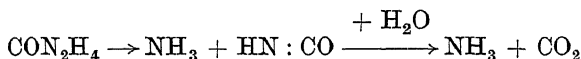
Cyanic acid is highly reactive and combines with the residual urea to form biuret, $\text{H}_2\text{N.CO.NH.CO.NH}_2$, which gives a rose colour with copper sulphate and an alkali.

(2) **Hydrolysis.**—Urea in sterile solutions is stable at ordinary temperatures, but if boiled alone, or in presence of alkalis or acids, it is converted eventually into ammonia and carbon dioxide.

The change, usually described as the hydrolysis of urea, is represented by the equation :—

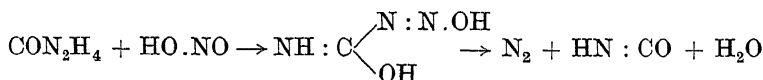


According to Werner, what happens is first the dissociation of urea into ammonia and cyanic acid, and then the hydrolysis of cyanic acid to ammonia and carbon dioxide.

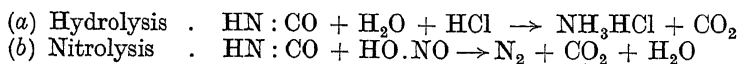


Urea solutions kept under ordinary laboratory conditions soon become alkaline owing to the action of micro-organisms. Until this was recognised it was accepted as an example of the spontaneous reversion of urea into ammonium cyanate.

(3) **Deamination by Nitrous Acid.**—The condition necessary for attack is the addition of a sufficiently strong acid (*e.g.*, HCl) to unmask the amide group, which undergoes (*a*) diazotisation, followed by (*b*) decomposition into nitrogen and cyanic acid.

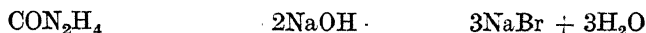


As it is generated, cyanic acid is decomposed by two independent reactions :—



The fixation of some of the cyanate nitrogen as ammonia and as a nitrate accounts for the yield of gaseous nitrogen invariably being less than the theoretical amount.

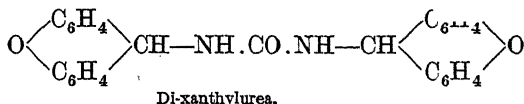
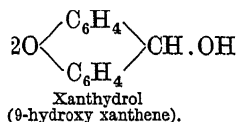
(4) **Deamination by Hypobromite.**—This reaction underlies the gasometric method of estimating urea. The condition necessary for attack is brought about by the addition of an alkali, which unmask the amide groups. Subsequent addition of hypobromite leads to bromination of the amide groups, followed by simultaneous hydrolysis and oxidation.



Owing to secondary reactions this change is never quantitative.

The chief secondary reactions are: (a) formation of cyanate and hydrazine; (b) formation of carbon monoxide. These three compounds have been detected by independent workers. The conditions governing their formation have been investigated by Werner.

(5) **Condensation with Xanthidrol.**—Xanthidrol condenses with urea in acid solutions to form an extremely insoluble di-xanthidylurea:



Owing to its low solubility, the xanthidrol is used as a 10 per cent. solution in methyl alcohol, and the urea solution must contain a large excess of glacial acid to keep the free xanthidrol from being precipitated by water, and also to unmask the amido groups so that they will react. The test will reveal urea in concentrations of 1 : 10,000 in about fifteen seconds; and 1 : 800,000 in ten minutes. A positive result is shown by a crystalline precipitate of di-xanthidylurea slowly appearing as a cloud of fine, colourless, silky needles.

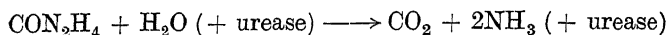
It is important to note that xanthidrol is unstable both in solid form and in solution, and soon loses its precipitating power. It should be prepared as required by the reduction of xanthone.

The reaction is not given by any of the natural amino acids, purines, pyrimidines, or simpler natural bases, and hence is of great biochemical value. Monosubstituted ureas, thiourea, urethane, and biuret, however, yield sparingly soluble condensation products.

The practical details of the technique are described by Fosse and by Werner.

(6) **Decomposition by Urease.**—Urea is rapidly and completely converted into ammonia and carbon dioxide by the widely distributed enzyme urease which is found in the seeds of many leguminous plants and in many micro-organisms. The chief sources of urease

are the seeds of the Jack Bean (*Canavalia ensiformis*), the Sword Bean (*C. gladiata*), and the Soy Bean (*Glycine hispida*). The optimal region of the reaction is about pH 7, and the change follows the simple equation :



The zymolytic decomposition of urea differs from the hydrolysis in that carbamic acid is the first identifiable product of the reaction (Sumner, 1926).

(7) **Analytical Reactions of Urea.**—(a) *Biuret Test.*—Solid urea is heated above its melting point for a few minutes. Fumes of ammonia are evolved, and a white deposit of ammonium cyanate condenses on the sides of the tube. After cooling, the residue is dissolved in water, and treated with sodium hydroxide and a few drops of dilute copper sulphate. A rose-pink colour shows the presence of biuret, which has been formed by union of the liberated cyanic acid with unchanged urea (p. 148).

(b) *Benzyldine Test.*—The solution is acidified with a few drops of HCl, and then a few drops of Ehrlich's aldehyde reagent (3 per cent. *p*-dimethylaminobenzaldehyde in 20 per cent. HCl) are added. An intense yellow colour develops if urea be present.

The colour is due to the formation of *p*-dimethylaminobenzyldine urea. Similar colours are given with compounds containing the —NH—CO—NH_2 grouping, such as biuret. Alkalies and excess of strong acid discharge the colour. Nitrites interfere by forming deep yellow nitro derivatives that are not bleached by alkalies.

(c) *Hypobromite Test.*—When alkaline sodium hypobromite is added to urea a vigorous effervescence occurs with liberation of free nitrogen. This reaction is widely used in the estimation of urea in urine, but its delicacy is limited by the fact that all the urea nitrogen is not evolved as gas, and also many other biological compounds, such as ammonia and amino acids, evolve nitrogen.

(d) *Urease Test.*—This is the most specific of all tests for urea. The neutral solution is incubated with a urease preparation and the liberated ammonia is detected by means of indicators, and estimated by titration or by colorimetry.

(e) *Xanthidrol Test*, which has been adapted for analytical use by Fosse (1928).

(f) *Diacetyl Test.*—A few drops of the urea solution are mixed with excess (2–3 ml.) of concentrated hydrochloric acid and 2–3 drops of 3 per cent. diacetyl monoxime. On boiling, a yellow colour develops if urea be present. The colour changes to deep orange on careful addition of an oxidiser (1–3 drops of 0.1 per cent. hydrogen peroxide or 1 per cent. potassium persulphate). Substituted ureas, including

citrulline, give red pigments (p. 150). The test is delicate and will reveal 0.1 mg of urea.

The Origin of Urea in the Animal Body : Ureagenesis

Ammonia is a characteristic end-product of amino acid metabolism. It is very poisonous, and must be detoxicated prior to excretion. In mammals, and many other types, this is done by conversion to urea, and excretion as a urinary solute. In birds and reptiles, ammonia is converted into the sparingly soluble uric acid, and thus excreted.

The adult human body, on an ordinary daily diet containing 80–100 gm of protein, manufactures and excretes upwards of 20 gm. of urea, *per diem*, which represents more than 11 gm. of ammonia. Consequently, *ureagenesis* is a process of the greatest importance. The mechanism is highly efficient, and there are no pathological records of its complete failure in any known disease.

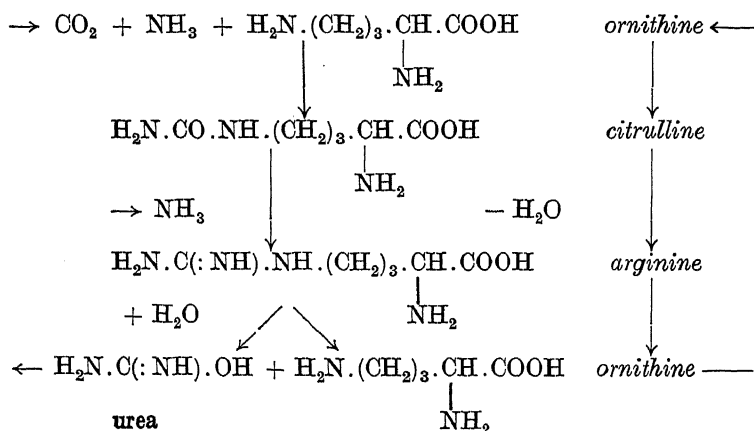
Location of Ureagenesis.—It has been suggested that ureagenesis is a property of most tissues, but perfusion and incubation experiments have shown that the mechanism is narrowly restricted to the liver, and there is no evidence that urea can be assembled from ammonia and carbon dioxide in any other tissue in the mammal, although the presence of arginase in the kidney may account for a slight subsidiary extra-hepatic production of urea from surplus arginine.

The unique significance of the liver was demonstrated experimentally in 1927 by Mann and his colleagues, who showed that total hepatectomy in dogs resulted in complete cessation of ureagenesis. The amino nitrogen and the ammonia of the blood, urine and tissues increased, while, at the same time, the urea of the blood and tissues decreased, owing to excretion in the urine. If the kidneys are ligatured, the blood urea remains constant in the hepatectomised animal.

Mechanism of Hepatic Ureagenesis. (1) *The Ornithine Cycle of Krebs and Henseleit* (1932). Although urease can produce urea from ammonia and carbonic acid by a reversion synthesis under suitable conditions, urease is absent from mammalian tissues, apart from traces found in gastric mucosa, and therefore cannot be concerned in ureagenesis. Arginase is the only enzyme known to liberate urea; and arginine was, until the recent discovery of canavanine, the only amino acid known to form urea on simple hydrolysis. In 1914, Clementi pointed out that arginase is present in the livers of all animals that excreted nitrogen as urea, but is absent from animals, such as birds and reptiles, that are *uricotelic* in that they excrete nitrogen as uric acid.

This suggested that arginase has a function in metabolism more important than the mere removal of surplus dietary arginine. The missing component in the system was provided by the discovery of citrulline, and the proof, in 1930, that it was a uramido acid midway in structure between ornithine and arginine. By employing a tissue-slice technique that enabled them to work with intact and actively respiring tissues, Krebs and Henseleit were able to show that the liver is the only organ capable of synthesising urea from ammonium carbonate, and, furthermore, that the rate of the synthesis was greatly increased by addition of one of the three amino acids, ornithine, citrulline or arginine. No increase in the rate of ureagenesis was observed on addition of any of the other natural amino acids or related compounds. In this process, the ornithine appeared to act as a catalyst or carrier, since it is not used up, and small amounts are able to bring about the synthesis of an indefinite quantity of urea, provided that ammonia and carbonic acid are continuously supplied.

From these facts, Krebs has explained urea formation in the animal body in terms of an ornithine cycle made up of three stages : (i.) Formation of *citrulline* by condensation of one molecule of ammonia and one of carbon dioxide with the δ -amino group of ornithine, (ii.) formation of *arginine* by condensation of a second molecule of ammonia with the citrulline, (iii.) decomposition of arginine by arginase, with formation of *urea* and *ornithine*, which rejoins the cycle.



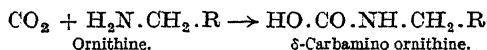
The Ornithine Cycle

The maximum yield of urea from the rat liver is about 4 per cent. of the dry tissue weight per hour, under normal conditions, but can

be increased three-fold by addition of citrulline. Urea synthesis is not necessarily accompanied by oxygen uptake, but can proceed anaerobically in presence of glucose, lactate or pyruvate as sources of energy.

At least three catalysts are required in the ornithine cycle, and of them, only one, namely *arginase*, has been separated, and will work in solutions; the others require the presence of intact liver tissue capable of respiration.

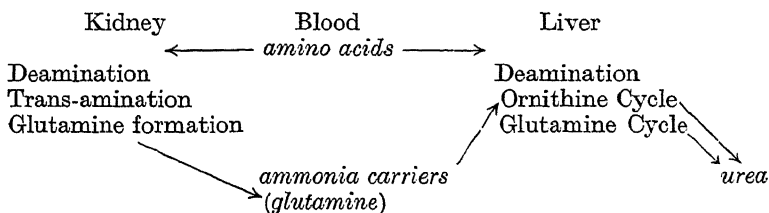
The obscure stages in the cycle are (i.) the conversion of ornithine into citrulline, and (ii.) the conversion of citrulline into arginine. According to Krebs (1936), the first of these depends on the combination between CO_2 and the δ -amino group of citrulline to form a carbamino compound,



The carbamino compound is then combined with ammonia, and dehydrated to form the uramido acid, citrulline, $\text{H}_2\text{N}.\text{CO}.\text{NH}.\text{R}$.

London and his colleagues (1937) were unable to confirm the operation of the Krebs cycle in the living animal, and report negative results following the perfusion with ornithine and citrulline. Ikeda (1938) claims that perfusion of dog liver by blood containing citrulline and ammonia results in formation of urea.

(2) *The Glutamine Cycle of Leuthardt* (1938).—In presence of liver tissue of cat, guinea-pig, or rat, glutamine produces 40–60 per cent. more urea than what would be obtained from an equivalent amount of ammonia. Unlike the ornithine cycle, the process is not dependent on tissue respiration, and free ammonia is not utilised. Leuthardt, the discoverer of the reaction, believes that it represents an independent and important mode of ureagenesis. The glutamine reacts with carbonic acid to form urea and pyrrolidine-2-carboxylic acid, which undergoes successive transformation to proline and glutamic acid, and subsequent amination to glutamine, thus completing the reaction cycle. Asparagine resembles glutamine in ureagenic properties.



Summary of the Ureagenic Systems

Significance of Urea in the Animal Body.—(1) *A Detoxication Product.* The primary function of urea is to provide a stable, inert

and very soluble form in which nitrogen can be eliminated; and thus removes the potentially toxic ammonia group from metabolic processes. The extent to which ammonia is converted into urea depends on the base-balance of the organism. In conditions of acidosis, ammonia may be diverted from ureagenesis, and used to depress the H-ion concentration of the tissues by combining with protons. This is shown by the fact that the excretion of ammonium salts in the urine is increased by acidogenic dietaries.

(2) *A Diuretic*.—During its renal excretion, urea carries with it sufficient water to keep its concentration below 2·5 per cent., and thus acts as a natural diuretic. This property is the basis of several tests for renal efficiency.

(3) *A Uræmic Toxin*.—Urea was formerly believed to be responsible for the pathological condition of *uræmia*, which is due to defective renal excretion and consequent rise in blood urea. Urea, however, is not an obviously harmful compound, and, although very marked hyper-uræmia is found in some forms of nephritis, it is probable that the toxic signs and symptoms are due to a *uro-toxæmia* from retention of other urinary solutes, of which ammonium ions are the most dangerous. Sustained hyper-uræmia may, however, give rise to changes in tissue proteins, and toxic metabolites may, in theory, be produced from urea. The opinion prevails that even if urea is not responsible for the uræmic syndrome, its concentration in the blood affords a good index of the severity of the disease.

The toxicity of large doses of urea is determined by the inability of the animal to excrete urea beyond a certain maximum rate which is about 1·5 gm. urea per 1 gm. kidney tissue per twenty-four hours, in the dog. Urea administered to rabbits in quantities of 0·1–0·2 per cent. of the total body weight evokes severe disturbance and death.

(4) *A Regulator of Osmotic Pressure*.—Urea is too soluble and diffusible to have an appreciable local effect on the osmotic pressure of the mammalian organism; and even when produced in quantity during protein metabolism, its removal by the kidney is so rapid that it can have little effect on the general osmotic pressure of the animal. The elasmobranchs, or cartilaginous fishes, such as the skate, dogfish and the shark, are unique in possessing in their blood, body fluids and tissues, concentrations of urea as high as 2–3 per cent. This physiological hyper-uræmia is due to the relative impermeability of the gills and the integument, and to the active conservation of urea by the elasmobranch kidney. As W. H. Smith has shown (1936), under these conditions, urea acts as a factor in the regulation of the osmotic pressure, and renders the elasmobranch osmotically superior to its environment, and able to

absorb a sufficient quantity of water from its marine environment to provide for the secretion of urine. Marine teleosts, or bony fishes, do not conserve urea, and obtain their necessary water supply by drinking sea water and excreting through their gills a solution having a higher sodium chloride content than that of their environment.

GENERAL REFERENCES

- BORSOOK, H., and C. JEFFREYS (1935), "Nitrogen metabolism in the rat." *J. Biol. Chem.*, **110**, 495.
- FEARON, W. R. (1926), "Biochemistry of urea." *Physiol. Rev.*, **6**, 397.
- FEARON, W. R. (1933), "Urea in health and disease." *British Med. J.*, *ii.*, 521.
- FEARON, W. R. (1936), "Structure of urea in reference to deamination by urease." *Biochem. J.*, **30**, 1652.
- FEARON, W. R. (1937), "Nitroferrocyanide reaction." *Analyst*, **62**, 586.
- FOSSE, R. (1928), "L'Urée." Paris.
- GUILLAUMIN, A. (1939), "Dérivés azotés de l'acide carbonique." *Traité de Chimie Organique*. Ed. Grignard. Vol. XIV. Paris.
- KREBS, H. A. (1934), "Urea formation in the animal body." *Ergebn. d. Enzymforschung*, **3**, 247.
- LEUTHARDT, F. (1938), "Harnstoffbildung aus glutamin." *Zeit. Physiol. Chem.*, **252**, 238.
- MCCANCE, R. A. (1930), "Chemistry of the degradation of protein nitrogen." *Physiol. Rev.*, **10**, 1.
- SIDGWICK, N. V. (1936), "Organic Chemistry of Nitrogen." London.
- SMITH, H. W. (1936), "Physiological role of urea in the elasmobranchii." *Biol. Rev.*, **11**, 49.
- WERNER, E. A. (1923), "Chemistry of Urea." London.

CHAPTER XXIII

EXCRETION

THE higher organism returns matter to the environment through four channels: the lungs, the skin, the intestine and the kidneys. Gaseous waste-products, notably carbon dioxide and water vapour, escape by the lungs; water and about 1 per cent. of solutes are lost in the perspiration; insoluble salts, food residues, mucin, and lipides are excreted by the intestine; and soluble salts and organic end-products are excreted in the urine.

From the clinical aspect, urine is most significant, since (with the exception of some ammonia and hippuric acid) every constituent has been derived from the blood, and has at one time formed part of the internal environment of the organism.

Average Composition of Normal Human Urine Solute excreted in Twenty-four Hours

Total volume . . .	1,250 ml.	pH 6.2.
Total solids . . .	58 gm.	
Total nitrogen . . .	17 gm.	Depends chiefly on protein intake.
Urea . . .	28 gm.	
Creatinine . . .	1.5 gm.	Independent of normal diet.
Uric acid . . .	0.7 gm.	Partly independent of diet.
Methyl purines . . .	0.1 gm.	Dependent on tea and coffee intake.
Hippuric acid . . .	0.65 gm.	Increased by fruit diet.
Amino acids . . .	+	Increased in wasting diseases.
Ammonia . . .	0.7 gm.	Increased in acidoses.
Indoxyl sulphate . . .	0.01 gm.	Depends on intestinal putrefaction.
Chloride, as NaCl . . .	12 gm.	Depends on salt intake.
Phosphate, as H_3PO_4 . . .	2.6 gm.	Depends on phosphoproteins of diet.
Sulphate, as H_2SO_4 . . .	2.0 gm.	Depends on proteins of diet.
Potassium . . .	1.6 gm.	Increased by vegetable diet.
Calcium . . .	0.2 gm.	
Magnesium . . .	0.2 gm.	Increased by vegetable diet.
Iron . . .	1-5 mg.	
Sugar . . .	0.7 gm.	Mostly pentose.
Oxalate . . .	0.01 gm.	Depends on diet and gastric fermentation of sugar.
Lactate . . .	+	Increased by muscular activity.
Thiol compounds . . .	0.3 gm.	Independent of diet.
Pigments . . .	+	

The composition of the urine varies during the day, owing to alterations in metabolic activity, and for comparative purposes the

twenty-four-hour output is taken as being representative. The extent to which individual variation can occur is shown in the following table compiled from data by Powell White (1925).

*Composition of Urine from 50 Normal Subjects
Expressed in mg. per 100 ml.*

Solute.	Average.	Maximum.	Minimum.
Urea N . . .	682	1,829	298
Urea . . .	1,459	3,914	638
Creatinine N . . .	36	90	17
Creatinine . . .	97.2	243	45.9
Uric acid N . . .	12.3	30.7	3.7
Uric acid . . .	36.9	92	11
Amino N . . .	9.7	42.5	0.6
Ammonia N . . .	57	189	13
Sodium . . .	212	608	46
Potassium . . .	137	245	56
Calcium . . .	19.5	72.5	0.6
Magnesium . . .	11.3	23.9	2.7
Chloride . . .	314	579	99
Total sulphate . . .	91	326	34
Inorganic sulphate . . .	83	304	32
Organic sulphate . . .	5.3	25.6	0.6
Inorganic phosphate . . .	179	426	77
pH . . .	6.4	8.2	5.8
Total acidity as ml. N/10 acid . . .	27.8	76.8	4.0

These data indicate the wide variations in urinary composition of British adults on varied dietaries.

According to Powell White (1925), the following correlations obtain in human urine.

Urea chiefly with creatinine, phosphate, potassium, and total acidity.

Creatinine chiefly with potassium, sodium, chloride, and water.

Uric acid chiefly with phosphate and total acidity.

Chloride chiefly with sodium and potassium.

Phosphate chiefly with potassium, calcium, and magnesium.

Sulphate chiefly with magnesium and potassium.

Ammonium chiefly with alkalinity.

Sodium and chloride have a high correlation with water, while sulphate is very low.

HUMAN URINE

(1) **Total Volume in Twenty-four Hours.**—Adult, 950–1,500 ml. (33–60 oz.); child, 400–600 ml. Night urine is about one-quarter to one-half the volume of day urine.

The total volume varies greatly with diet and season of the year.

In polyuria, such as diabetes insipidus due to lack of the anti-diuretin autacoid of the pituitary gland, the daily output of urine may be as great as 10 litres, and is balanced by a corresponding thirst. In oliguria, the output may be only a few ml., or nil in complete suppression (anuria).

(2) **Density.**—1,015–1,020 at 15° C. (water = 1,000). The density is a measure of the concentration of urinary solutes, and is inversely related to the total volume. After drinking an excess of aqueous fluids, the density may be as low as 1.002; whereas, after twelve hours' abstention from fluids, it may rise to 1.030–1.035.

In determining the density, the urine should be at room temperature (15° C.), and the hydrometer floating freely in the liquid. Froth on the surface can be removed by a drop of alcohol.

(3) **Appearance.**—Fresh urine is usually transparent, but may be opaque after meals owing to the "alkaline tide" causing a precipitation of calcium and magnesium phosphate (p. 395). As urine cools, a cloudy suspension of mucoprotein from the urinary tract may appear in the body of the fluid.

(4) **Colour.**—The normal colour of urine is due chiefly to two pigments:—

- (a) *Urochrome*, a stable yellow pigment invariably present in urine, and derived probably from pyrrole metabolism (p. 186). It has no characteristic spectrum, and its excretion has not been correlated with any particular metabolic conditions. Urochrome can be obtained in a very impure form by extracting acidified urine with amyl or butyl alcohol.
- (b) *Urobilin*, a brown pigment excreted as a colourless precursor, urobilinogen, which gradually oxidises on exposure to light and air. Urines rich in urobilinogen darken on standing owing to this spontaneous oxidation.

Urobilinogen is derived from bilirubin that has undergone reduction in the intestine, and its excretion is an index of the degree of intestinal stasis and putrefaction. It disappears from the urine in

obstructive jaundice, being replaced by bilirubin excreted directly from the plasma, but it reappears in an increased amount during the recovery stage. Urines containing urobilinogen give a red colour on addition of a few drops of an acid solution of Ehrlich's aldehyde reagent (p. 149), without application of heat. Urobilin does not give this reaction, but may be detected by its characteristic spectrum (p. 186), and by the green fluorescence developed on addition of a few drops of a 2 per cent. solution of zinc acetate in alcohol. Minor pigments present in urine are *uroerythrin*, which imparts a red colour to uric acid deposits, and *uroporphyrin*, a solute greatly increased in the congenital condition of porphyrinuria.

(5) **H-ion Concentration.**—Urine is usually slightly acid in reaction, the average value being pH 6.0, but it may vary from pH 4.8–pH 7.5 during the twenty-four hours. After rising in the morning, and about half an hour after each meal, the reaction of urine tends to shift towards alkalinity, the so-called “alkaline tide.” This promotes the precipitation of calcium and magnesium phosphates.

The H-ion concentration of urine may be determined electrometrically or colorimetrically by means of selected indicators covering the range pH 4–pH 8.

(6) **Total or Titration Acidity.**—This is found by titrating 10 ml. of urine with N/10 NaOH, using 2 drops of 0.1 per cent. phenol-red or phenolphthalein as indicator. The result is expressed as the quantity of N/10 NaOH required to neutralise (1) 100 ml. urine, or (2) the total volume of urine excreted in twenty-four hours.

Titration is a measure of the available acidity of urine; this depends chiefly on the concentration of acid phosphate ions, and also on the uric and hippuric acid present. Usually 200–500 ml. N/10 NaOH are required to neutralise the acidity of an entire twenty-four hour sample.

To determine the end-point, compare the urine being titrated with an untitrated specimen in a similar test tube. As soon as a colour difference can be detected the titration is complete. Shortly before the end-point is reached, a precipitate of calcium and magnesium phosphate may appear in the solution. This is due to the conversion of soluble acid phosphate into insoluble phosphate by action of the alkali. Folin advocates the precipitation of the calcium by the addition of neutral potassium oxalate before starting the titration.

INORGANIC SOLUTES

Ammonia.—The average percentage of ammonia is 0.05, corresponding to a daily output of 0.7 gm. It is present as NH_4^+ , unless

the urine has undergone fermentation, when free ammonia may be detected. Urinary ammonia arises partly by a diversion of the amino acid nitrogen from urea formation, and partly by local synthesis in the kidney. It is greatly increased in all conditions of acidosis, being part of the defence mechanism of the organism.

Tests for Ammonia.—(1) *Nessler's Reaction.*—To 5 ml. of water add a few drops of Nessler's reagent. There is no colour change if the water be free from ammonia. Now add a drop of urine. An intense yellow colour develops owing to the ammonia present in the urine. (2) Heat 5 ml. of urine with a little solid sodium carbonate. Ammonia is set free, and can be detected by the smell and by the bluing of red litmus paper held above the tube.

Estimation of Ammonia.—Neutralise 10 ml. of urine to phenolphthalein, using N/10 NaOH, as in the estimation of the total acidity of urine. Neutralise 2–3 ml. of commercial formalin solution (30–40 per cent. formaldehyde) in the same manner. Mix the two neutral solutions. The mixture becomes acid, and the pink colour of the indicator is discharged. This is because the H-ions previously combined with the ammonia have been liberated by the formaldehyde. Titrate this increase in acidity as before with N/10 NaOH. Each ml. of alkali required corresponds to 1.7 mg. of NH_3 in the original urine. The ammonia value obtained by the formaldehyde method is usually 10–25 per cent. too high owing to the fact that the $-\text{NH}_2$ groups of other urinary solutes also interact. The discrepancy is usually neglected in clinical estimations.

Urinary ammonia may be estimated accurately by (a) aspiration and (b) by colorimetry, using Nessler's reagent.

The average ammonia output in twenty-four hours is about 0.7 gm., corresponding to 400 ml. N/10 NaOH. It is increased in conditions of acidosis, and by dietaries rich in protein.

Chloride.—Next to urea, chlorides are the chief solutes of urine. Expressed as NaCl, the average daily excretion is 10–15 gm., and the urinary concentration is about 0.7–1.0 per cent.

Silver Test for Chlorides.—Add 1 ml. of 1 per cent. AgNO_3 to 5 ml. of urine. A whitish precipitate forms, made up of silver chloride, carbonate, and phosphate. Acidify with about 10 drops of concentrated HNO_3 . Carbonate and phosphate dissolve, leaving a white residue of AgCl .

Estimation of Chloride by the Tartrazol Method.—Transfer by means of a pipette 10 ml. of N/10 AgNO_3 to a 100 ml. measuring cylinder. Acidify with 5 drops of concentrated HNO_3 . Add 2–4 drops of 0.5 per cent. tartrazine (tartrazol, or "tartar yellow"). Titrate with the urine, or other chloride solution, from a burette, by additions of 0.2 ml. at a time. Shake the yellow mixture vigorously

after each addition of chloride. The first effect of the chloride is to form a white precipitate of silver chloride, this rapidly adsorbs the indicator, and settles down as a buff coloured deposit. The surrounding liquid becomes colourless owing to removal of the tartrazol.

The end-point is reached when all the silver has been precipitated as chloride. At this stage, addition of a slight excess of chloride causes a sudden release of the adsorbed indicator. The colourless solution turns bright yellow, and the silver precipitate changes from buff to pure white. The estimation should be done in duplicate as the end-point is very sharp, and may be overshoot the first time.

Calculation.—Since 10 ml. N/10 AgNO_3 = 58 mg. NaCl, $x = \frac{5.8}{n}$,

where n = number of ml. of chloride solution added,

and x = percentage of chloride, expressed as gm. NaCl per 100 ml.

The usual value for n is 5–9, corresponding to an NaCl content of 1.16–0.63 per cent.

If the chloride content of the urine is less than 0.1 per cent., use 1 ml. of N/10 AgNO_3 in a test tube, and add 1 drop of indicator.

Proteins in pathological urine interfere with the end-point by inhibiting the aggregation of the AgCl , but do not affect the colour change.

Phosphate.—(a) *General Test.*—Acidify 5 ml. of urine with about 10 drops of concentrated nitric acid. Add 2 ml. of 2 per cent. ammonium molybdate ($(\text{NH}_4)_2\text{MoO}_4$), and boil gently. A bright yellow precipitate of phosphomolybdic acid forms if phosphate be present.

(b) “*Earthy Phosphates.*”—Make alkaline 5 ml. of urine with about 10 drops of ammonium hydroxide. A cloudy precipitate of calcium and magnesium phosphates (“earthy phosphates”) forms. Heat the mixture. The precipitate flocculates. Acidify with 20 per cent. acetic acid. The precipitate dissolves.

The precipitation of these phosphates when urine is boiled is a common fallacy in the heat-coagulation test for proteins in urine. They can be distinguished from proteins by their solubility in acids. Earthy phosphates tend to precipitate whenever the H-ion value of urine falls below pH 6, and thus they appear during the “alkaline tide” after meals.

Estimation of Phosphate.—Urinary phosphate may be rapidly and roughly estimated by means of uranium acetate. Measure 1 ml. of an accurately prepared 3.50 per cent. uranium acetate solution into a test tube. Dilute with 3–4 ml. water. Add 2–3 drops of 5 per cent. potassium ferrocyanide. A brown precipitate

of uranium ferrocyanide forms. Boil the mixture, and titrate with urine from a burette, while boiling, until the brown colour is completely discharged.

Calculation.—Since 1 ml. of the standard uranium acetate,

$$(\text{CH}_3\cdot\text{COO})_2 : \text{UO}_2\cdot 2\text{H}_2\text{O} = 0\cdot005 \text{ gm. } \text{P}_2\text{O}_5, \text{ or } 0\cdot0035 \text{ gm. } \text{H}_3\text{PO}_4,$$

where n = number of ml. of urine required,

$$x = \frac{0\cdot35}{n}, \quad \text{and } x = \text{phosphate percentage, expressed as gm. } \text{H}_3\text{PO}_4 \text{ per 100 ml.}$$

The daily phosphate output, expressed as the acid, H_3PO_4 , is about 2·6 gm. It is largely derived from the phosphoproteins of the diet.

Sulphate.—(a) *Inorganic.*—Add a few drops of 2 per cent. BaCl_2 to 5 ml. of urine. A white precipitate of BaSO_4 forms. This does not dissolve on addition of strong nitric acid, thus being distinguished from BaCO_3 and $\text{Ba}_3(\text{PO}_4)_2$, which are also precipitated on addition of BaCl_2 .

(b) *Organic.*—Add an excess (2–3 ml.) of BaCl_2 to 5 ml. of urine. Filter off the precipitated sulphate, carbonate, and phosphate, repeating the filtration, if necessary, to obtain a clear filtrate. Acidify the filtrate with a few drops of nitric acid, and boil for a few minutes. Gradually a second precipitate of barium sulphate forms, owing to the hydrolysis of various organic sulphates in the urine. These are compounds of indoxyl and other phenols, and are sometimes termed “ethereal sulphates.”

The total daily sulphate output, expressed as H_2SO_4 , is about 2 gm., and is derived from the sulphur-containing amino acids, cystine and methionine, of the protein dietary. About 80 per cent. of total sulphate is inorganic, and 20 per cent. is organic, arising from the detoxication of phenols produced during the putrefaction of proteins in the digestive tract.

Urinary sulphur also appears in a third form, sometimes termed “neutral sulphur.” This includes thiol compounds, such as $\text{CH}_3\cdot\text{SH}$, and thiocyanate, which do not interact with the barium reagent until they are oxidised to sulphate.

ORGANIC SOLUTES

Urea, $\text{H}_2\text{N}\cdot\text{C}(\text{NH})\cdot\text{OH}$, the chief nitrogenous constituent of mammalian urine, is derived from the protein of the diet in the proportions of about 30 gm. urea per 100 gm. of protein. The daily output is 25–32 gm., and the urinary concentration is usually in the region of 2 per cent.

TESTS: (1) *Zymolysis by Urease.*—To 5 ml. urine add a little

urease preparation (soy bean powder or extract) and 5 drops of phenol red or "universal" indicator. If the mixture be not acid to the indicator (*i.e.*, yellow to phenol red at the start), carefully acidify with a weak acid (0.2 per cent. acetic). Incubate at 40–50° C. or keep at room temperature. The presence of urea is shown by the mixture gradually becoming alkaline owing to liberated NH_3 .

(2) *Decomposition by hypobromite*.—To 5 ml. urine add about 1 ml. fresh hypobromite (made by adding 25 ml. Br. to 250 ml. 40 per cent. NaOH). The urea is decomposed with liberation of free N_2 , and the mixture effervesces vigorously. Ammonium salts, amino acids, and other compounds containing the *amino group* $-\text{NH}_2$ are decomposed in the same way.

(3) *Aldehyde Test*.—Add 3–5 drops of Ehrlich's aldehyde reagent (p. 149) to 2 ml. of water. Acidify with 3–5 drops of concentrated hydrochloric acid. On addition of a few drops of urine the mixture turns deep yellow owing to formation of a pigment from the urea and the aldehyde.

Nitrites, which appear in stale urine, interfere with the test by forming yellow nitro derivatives, which, unlike the urea pigment, are not bleached by addition of alkali. Sulphanilamide, which may appear in the urine after administration of one of the many forms of the drug, gives an intense red colour with the reagent, and thus may be estimated colorimetrically (A. E. Werner, 1938).

Allantoin gives a colour similar to urea, but never appears in sufficient quantities in human urine to respond to the above form of the test.

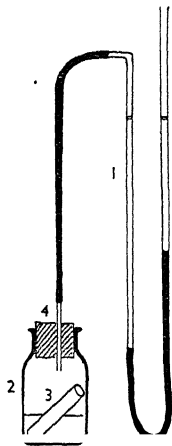
(4) *Diacetyl Test*.—Apply this test to 2–3 drops of urine mixed with 2–3 ml. HCL (p. 384).

Estimation of Urea.—(a) *Decomposition by Urease*.—This is employed in the standard methods for the accurate estimation of urea in blood, urine and other biological liquids. The urea is converted into ammonium carbonate, which is estimated directly by Nessler's reagent, or aspirated into standard acid (Marshall and Van Slyke, 1915), or allowed to diffuse into standard acid (Conway, 1933).

(b) *Decomposition by Hypobromite*.—By measuring the nitrogen evolved as gas when urea is decomposed by hypobromite in presence of alkali, which fixes the carbon dioxide, a value is obtained which is sufficiently accurate for clinical estimations. Many different types of apparatus are in use; the one now described can be improvised from a couple of 10 ml. pipettes, and is easy to assemble.

- (1) *Pipettes filled with water coloured with methylene blue.*
- (2) *Outer flask for hypobromite.*
- (3) *Test tubes for urea solution.*
- (4) *Glass tube, which can be moved up and down.*

Place 10 ml. of the hypobromite reagent (p. 397) in the outer flask, and dilute with about 10 ml. of water. Measure, by pipette, exactly 1 ml. of urine into the small test tube, and insert it in the flask so as not to mix the liquids. Cork the flask securely. Disconnect the rubber tube from the glass tube. Adjust the water levels in the pipettes so that they are equal. Reconnect the rubber tube, and by moving the glass tube up or down readjust the water levels, if necessary. Tilt the flask so as to mix the liquids, and shake gently to expel the nitrogen. Let the flask cool for a couple of minutes. Adjust the water levels to the same height, by moving the pipettes. Take the reading of the left-hand pipette as soon as the gas evolved has become constant in volume.



Calculation.—In theory, 373 ml. of nitrogen at n.t.p. are evolved by 1 gm. of urea, but experimentally it is found that about one-twelfth of this is not liberated when hypobromite acts on urea in concentrations of 1–3 per cent., which covers the range of concentrations found in normal urine. This deficit is partly compensated by the evolution of nitrogen from other amino compounds in the urine, and in practice it is assumed that 1 ml. of $N_2 = 2.8$ mg. urea, at n.t.p.

Hence, $x = 0.28 v$, where v is the volume in ml. of gas evolved, and x is the percentage of urea.

For more accurate work it is necessary to correct for temperature as follows:—

$$x = 0.28 v \times \frac{273}{(273 + t)}, \text{ where } t \text{ is the centigrade temperature.}$$

Uric Acid, $C_5H_4N_4O_3$, the least soluble organic constituent of urine. Average value, 0.04 per cent., or 0.6–0.9 gm. in twenty-four hours. It is an end-product of nucleoprotein metabolism.

Phosphotungstate Test.—Saturate 5 ml. urine with sodium carbonate, or add 10 drops of urine to 10 ml. saturated sodium carbonate solution. Then add about 1 ml. of Folin's uric acid reagent; an intense blue colour develops approximately proportional to the amount of uric acid present.

Creatinine, $C_4H_7N_3O$, the anhydride of creatine. A constant and characteristic constituent of mammalian urine. The adult daily output is about 1.2 gm., and is independent of the diet.

TESTS: (1) *Picric Acid*.—Add 5 drops of saturated picric acid solution to 5 ml. urine. Make alkaline with a few drops of 20 per cent. NaOH. A deep orange colour develops proportional to the amount of creatinine present.

(2) *3,5-Dinitrobenzoic acid*, similarly applied, gives a purple colour with creatinine in alkaline solution. The reagent is more specific than picric acid.

(3) *Nitroprusside*.—Add 5 drops of fresh 5 per cent. sodium nitroprusside solution to 5 ml. urine. Make alkaline with a few drops of 20 per cent. NaOH. A deep ruby colour develops if creatinine be present. Acidify with 20 per cent. acetic acid; the colour is discharged.

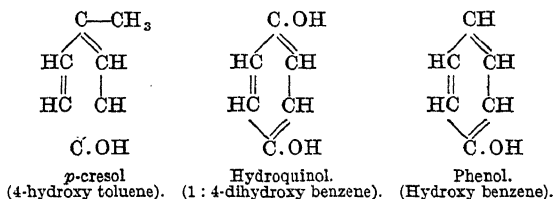
Note.—Acetoacetic acid and acetone, the pathological solutes found in ketosis, give a similar reaction with alkaline nitroprusside, but the colour is not discharged on acidification with acetic acid.

Hippuric acid, or benzoyl glycine, $C_6H_5.CO.NH.CH_2.COOH$, is synthesised in the kidney and in the liver from benzoic acid and glycine. The adult daily output is about 0.7 gm., being derived mostly from benzoic precursors in the vegetable diet.

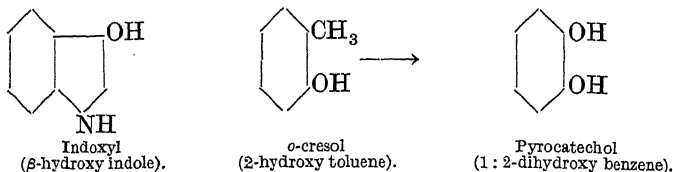
Urine is concentrated to one-quarter of its volume, filtered, acidified with H_2SO_4 and treated with $(NH_4)_2SO_4$ so as to make a 3 per cent. solution. On standing for twenty-four hours, hippuric acid crystallises out in four-sided prisms, which may be purified by recrystallisation from hot water. A good yield is obtained from the urine of herbivora, such as the horse or the cow.

Phenols.—Aromatic hydroxy compounds mostly derived from tyrosine and tryptophane putrefaction occur in the urine in normal health. The adult value on a mixed diet ranges from 20–60 mg. *per diem*. but is greatly increased in intestinal stasis and excessive protein dietaries.

(a) *Phenols derived from phenyl alanine and from tyrosine* :—

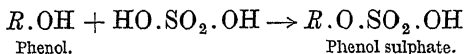


b) Phenols derived from tryptophane :—



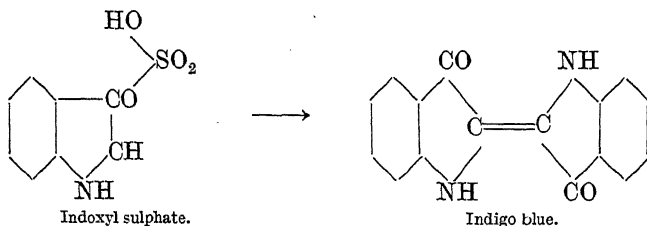
Various intermediate products derived from tryptophane have been reported as occasional urinary constituents. These include indole aldehyde, indole acetic acid, scatoxyl, and indole carboxylic acid. Indole and scatole, themselves, are improbable urinary constituents.

Urinary phenols are excreted as esters of sulphuric acid, in which form they are non-toxic.



(a) *General Test for Phenols.*—To 5 ml. of urine add Millon's tyrosine reagent drop by drop as long as a white precipitate forms. The precipitate settles out, and slowly turns red owing to the interaction between the adsorbed phenols and the reagent.

(b) *Indigo Test for Indoxyl.*—To 5 ml. of urine add an equal volume of concentrated HCl, and a drop of dilute (3 per cent.) hydrogen peroxide. Shake well. If the urine contains an excess of indoxyl it will be converted into indigo blue, and the urine will turn a dark bluish colour. Traces of blue may be detected by extracting the urine with chloroform, which dissolves out the pigment.



Three stages are recognisable in this curious test : (i.) hydrolysis of indoxyl sulphate and liberation of free indoxyl by the action of the acid ; (ii.) union between two molecules of indoxyl to form the leuco-base, or precursor of indigo blue ; (iii.) oxidation of the leuco-base to form the pigment. If the oxidiser be in excess a fourth stage terminates the reaction, namely, (iv.) oxidation of the indigo blue to the almost colourless isatin.

The test is unreliable, and sufficiently insensitive to be a controversial method for detecting abnormal amounts of indoxyl in urine.

In the absence of an oxidising agent the blue colour will not develop; in the presence of an excess of oxidiser the blue colour will be destroyed. Concentrated HCl has varying oxidising properties according to the amount of decomposition that has taken place owing to exposure to light. A yellowish specimen that contains oxides of chlorine is usually sufficiently active to give the indigo reaction without addition of H_2O_2 .

Many constituents in urine interfere with the test by combining with the liberated indoxyl.

(c) *α -Naphthol Test for Indoxyl.*—To 1 ml. of urine add 2–4 drops of 2 per cent. α -naphthol (or 2 per cent. resorcinol, or 2 per cent. thymol), and 10 ml. of concentrated hydrochloric acid. Shake well. If a colour does not develop in a minute, add a drop of 3 per cent. hydrogen peroxide. The liberated indoxyl combines with the α -naphthol to form a deep blue pigment (with resorcinol and with thymol, the colour of the pigment is carmine). This test is so delicate that all specimens of normal urine respond more or less.

PATHOLOGICAL CONSTITUENTS OF URINE

While a discussion of the various pathological constituents of urine and their significance is outside the scope of this book, the occurrence and identification of several of them presents features of biochemical interest, and they will be described briefly.

Pathological constituents may be divided as follows:—

(a) Tissue components and metabolites that normally do not appear in the urine.

Examples are serum albumin and serum globulin, hæmoglobin, bile pigments, bile salts, blood sugar.

(b) Abnormal or exceptional metabolites that escape by the urine.

Examples are β -hydroxy-butyric acid, acetoacetic acid, acetone, homogentisic acid, Bence-Jones protein, pentoses, lactose, methæmoglobin, hæmatoporphyrin.

(1) **Proteinuria.**—Normal urine contains a trace of protein, probably derived from the urinary tract. Under abnormal renal conditions the serum proteins may appear, giving rise to **albuminuria**. Again, in disease of the kidney and other tissue, protein decomposition products may escape and cause **proteosuria**. While in multiple osteo-myelomata, skeletal material may be excreted as “Bence-Jones protein.”

Usually, however, the term proteinuria denotes albuminuria, the appearance of a heat-coagulable protein in the urine.

TESTS: (1) *Heat coagulation.*—Transfer about 5 ml. of urine to a test tube, and carefully boil the upper layer. If the urine contains albumin a white coagulum may appear in the heated part of the tube. This must not be confused with the white, cloudy

precipitate of calcium and magnesium phosphates ("earthy phosphates") that forms when most specimens of normal urine are boiled. To distinguish, acidify the urine with a few drops of dilute acetic acid. The phosphate precipitate dissolves completely, the protein coagulum remains.

Urine that has become strongly alkaline owing to ammoniacal fermentation must be acidified slightly with acetic acid before the presence of protein can be detected by the heat-coagulation test.

Heat coagulation requires slight acidity and an electrolyte. Normal urine may provide the latter by its 1 per cent. content of NaCl, but urine from subjects of chloride retention may be so deficient in NaCl that any protein present will not coagulate on heating. To avoid this serious fallacy the modified form of the heat coagulation test has been devised, and should be used in all routine urine analysis.

(2) *Modified Test.* To 10 ml. urine add 1 ml. of the protein reagent (12 per cent. sodium acetate in 5 per cent. acetic acid), and boil for thirty seconds. If the urine remains clear, either it contains no coagulable protein or less than 5 mg. per 100 ml.

Bence-Jones Protein occurs in the urine in conditions of multiple myeloma of bone marrow, in myeloid and lymphatic leucæmia, and, occasionally, after severe fractures. The protein has been obtained from bone marrow by Meyler (1936), who suggests that it is in some way associated with the growth and activities of the leucocytes.

This form of proteinuria was recognised by Bence-Jones from the fact that on heating the urine the protein began to flocculate at the relatively low temperature of 40° and re-dissolved on heating to 100°. On cooling, the protein precipitate reappears.

(3) *Nitric Acid Precipitation* (Heller's Test).—Place 2–3 ml. of concentrated nitric acid in a test tube and carefully add about 5 ml. of urine by means of a pipette so as to form a layer above the denser acid. An opaque white ring or cloud at the junction of the liquids denotes albumin.

(4) *Salicyl Sulphonic Acid Precipitation.*—To 3 ml. of urine add about 6 drops of 20 per cent. salicyl sulphonic acid. If the urine contains more than 0.02 per cent. of albumin a white cloud forms almost at once and becomes more dense. Large quantities of protein give an opaque white precipitate.

The modified heat coagulation test and the salicyl sulphonic acid test are each about four times as sensitive as the nitric acid test.

Positive results with these tests do not differentiate between albumin and globulin in the urine. This can be done by fractional precipitation by neutral salts, but the distinction has not yet been shown to have much clinical significance. Globulin always accompanies albumin in albuminuria, though the ratio may vary.

Note.—All three protein tests should be applied in the routine examination of clinical specimens. To detect a faint precipitate in any one of the tests, hold the tube against a dark background, and compare the contents with some of the untreated specimens.

(2) **Hæmaturia.**—Blood may appear in the urine in the form of corpuscles, free hæmoglobin (hæmoglobinuria), and abnormal derivatives (methæmoglobin, and hæmatoporphyrin). In hæmaturia the urine often has a smoky, reddish colour that is very characteristic. Microscopic examination of the sediment may show the presence of blood corpuscles if they have escaped hæmolysis.

TESTS: (1) *Benzidine Test.*—Add about 10 drops of a fresh, strong solution of benzidine in glacial acetic acid to 1 ml. of 3 per cent. (10 vol. O_2 per 100 vol.) hydrogen peroxide. Add 1 ml. of urine, and mix well. If blood be present the mixture turns dark blue.

(2) *Guaiacum Test.*—Dissolve a small fragment of guaiacum resin in about 3 ml. of alcohol, with the aid of heat. Cool. Add 10 drops of 3 per cent. hydrogen peroxide and 1 ml. of urine. Mix well. If blood be present the mixture turns blue.

(3) *Pyramidon Test.*—Mix about 2 ml. of urine with an equal volume of 5 per cent. pyramidon (amidopyrine) in alcohol. Acidify with 2 drops of glacial acetic acid. Add 2–4 drops of 3 per cent. hydrogen peroxide. Mix. If blood be present a lilac colour develops.

Notes.—These oxidation tests for blood depend on the presence of the heat-stable catalyst hæmoglobin, which they will reveal in dilutions as low as 1:10,000 to 1:50,000 in urine, and less than 1:200,000 in water.

A heat-labile catalyst, or enzyme, occurs in leucocytes, fresh milk and unboiled plant extracts, and will give a positive reaction with the reagents used in testing for blood. To distinguish, boil a sample of the urine, and repeat the test after cooling. If the response is no longer positive it was originally due to an enzyme.

The presence of leucocytes or pus in urine (pyuria) is most easily confirmed by microscopical examination of the sediment.

Urine after the administration of iodides usually gives a positive reaction with the blood reagents, and this may readily be mistaken for hæmoglobin as both reactants survive boiling previous to testing.

To distinguish, apply the spectroscope, and examine for absorption bands of blood pigment; and also centrifuge the specimen, and examine microscopically for unhæmolysed red corpuscles.

(4) *Spectroscopic Test.*—This may require special treatment of the urine, as the concentration of pigment is often insufficient to show the characteristic absorption spectra (p. 195).

The urine is acidified with acetic acid and extracted with an equal volume of ether. This dissolves out the hæmatin, which may be

extracted with dilute ammonium hydroxide, and identified spectroscopically.

Hæmaturia is a serious condition, and the analysis for blood in the urine should be confirmed by more than one test.

(3) **Biliuria.**—Bile constituents may appear in the urine in obstructive jaundice (overflow biliuria), toxic jaundice, and various hæmolytic disorders leading to the decomposition of hæmoglobin.

In simple obstructive jaundice bilirubin appears, accompanied later by the bile salts. In the other forms of biliuria, pigments are present unaccompanied by bile salts.

Bile Pigments

Urine containing bile pigment (bilirubin) has a characteristic golden-brown colour and shows a transient yellow froth on being shaken.

(1) *Iodine Test.*—To 5 ml. of urine carefully add about 10 drops of 1 per cent. iodine in alcohol (tincture of iodine) so as to form a layer on the surface. A green ring gradually develops between the layers if the urine contains bilirubin. The test may also be carried out by putting a drop of iodine solution on a drop of urine spread on a filter paper.

(2) *Turpentine Test.*—Acidify 5 ml. of urine with about 10 drops of glacial acetic acid. Add 2 ml. of turpentine, shake so as to form an emulsion, and warm gently. Do not boil, or the mixture may spurt.

If bile pigment be present, the white turpentine emulsion gradually turns green. Eventually a layer of turpentine separates out on top; this is colourless in simple obstructive jaundice, but is greenish in the toxic and hæmolytic forms of biliuria.

The reason appears to be that in obstructive jaundice the overflow pigment appears in the urine in an esterified form, insoluble in warm turpentine. In toxic jaundice this esterification has not taken place, and the pigment is turpentine-soluble.

Bile Salts

(3) *Sulphur Sedimentation* (Hay's Test).—Sprinkle the surface of 10 ml. of urine with some finely powdered sulphur. In normal urine the particles remain on the surface supported by the tension and the urine-air interface. If, however, the urine contain bile salts, the interfacial tension is reduced, and the sulphur particles gradually sink through the liquid. A control test should be done at the same time, using 10 ml. of water.

(4) *Peptone Precipitation* (Oliver's Test).—Filter the urine, if

necessary. Acidify 5 ml. with a few drops of glacial acetic acid, and add about 2 ml. of a clear 1 per cent. solution of peptone previously acidified with acetic acid. If bile salts be present a white precipitate forms.

These two tests illustrate two important properties of bile salts; reduction of surface tension, and precipitation of peptone. They are not as useful clinically as the tests for bile pigment.

(4) **Glycosuria.**—Normal urine contains small quantities of reducing sugars, the concentration being between 0.01 and 0.1 per cent., which is insufficient to affect the ordinary Benedict and Fehling tests. These sugars consist largely of pentoses and disaccharides, with a trace of glucose.

(1) *Benedict's Qualitative Test.*—Add 0.5 ml. of urine (8 drops, not more) to 5 ml. of Benedict's qualitative reagent. Mix, and heat over a small flame for one to two minutes, or, much better, immerse the tube in boiling water for two to three minutes (this avoids spurting of the mixture). Allow to cool for a few minutes, and observe the appearance.

A *positive result* is shown by a greenish turbidity with a yellow or red sediment. A slight yellow precipitate indicates 0.1–0.25 per cent. of sugar; a dense orange-red precipitate and a clear supernatant liquid indicates over 1.5 per cent. of sugar.

A *negative result* is shown by the solution remaining a clear blue, with possibly a grey precipitate due to excess of urates.

If excess of urine be added or if boiling be prolonged a positive result may be obtained with many specimens of normal urine owing to the traces of sugars they contain. For this reason, the technique of the test must be followed carefully.

A positive result with Benedict's reagent indicates the presence of one or more of the following pathological urinary constituents: glucose, lactose, pentose, fructose, glycuronic acid.

Glucose is by far the commonest reducing sugar found in urine; it may be identified by the osazone test (p. 115), and by yeast fermentation (p. 113), and verified by the observation of an accompanying hyperglycæmia.

Lactose is a normal constituent of most urines during the lactation period, and has no pathological significance. It can be distinguished from glucose by its non-fermentability by yeast, and by the fact that it does not yield a glucosazone.

Lactosazone in crystalline form can only be obtained with difficulty from urine containing lactose.

L-Xyloketose, the commonest urinary pentose, may be identified by the fact that it can reduce Benedict's reagent at temperatures much below boiling.

Reduction test for pentose and fructose (Lasker and Enklewitz, 1933) : Mix 1 ml. of urine and 5 ml. of Benedict's qualitative reagent, and incubate at 55° C., for ten minutes. The appearance of a yellow precipitate indicates that the urine contains L-xyloketose or fructose. Fructosuria, which is very rare, may be detected by the fact that the urine gives a positive fermentation test and yields glucosazone.

(2) *Fehling's Test*.—Boil equal volumes (3–5 ml.) of Fehling's mixed reagent and urine in separate tubes. If spontaneous reduction occur in the reagent, it must be discarded. When boiling, add the reagent in small quantities to the urine, and look for the colour change to orange that denotes reduction. The commoner fallacies of the test are described on p. 112.

(3) *Yeast Fermentation Test*.—The method is described on p. 114. Lactose, pentoses and glycuronic acid are the only non-fermentable copper-reducing substances likely to occur in urine, and the fermentation test should be used to check the tests of Benedict and Fehling.

(4) *Osazone Test*.—The phenylhydrazine reagent (p. 115) will readily detect glucose in urine down to concentrations of about 0.5 per cent., but is not so satisfactory in the detection of lactose, owing to the interfering effect of other urinary solutes.

Glycuronic acid, $\text{HOOC} \cdot (\text{CHOH})_4 \cdot \text{CHO}$, occurs in urine as a detoxication compound formed after the administration of chloral, camphor, naphthol, menthol, phenol, morphine, turpentine, anti-pyrin, aspirin, and other drugs, with which it is esterified.

The formation of glycuronates is regarded as a test of hepatic efficiency, and may be invoked by administration of 5 grains of aspirin by the mouth. Glycuronic acid reduces Benedict's and Fehling's reagents and is not fermentable by yeast.

Naphthoresorcinol Test (Tollens).—Add 5 ml. concentrated HCl and 10 drops of 1 per cent. alcoholic naphthoresorcinol to 5 ml. of urine. Mix, and heat to boiling. Boil for one minute, shaking at intervals. A red colour develops. Let the tube stand for five minutes, cool under the tap, and extract with 5–10 ml. of ether. If glycuronic acid be present the pigment dissolves in the ether to form a purple solution showing two absorption bands, one on the D-line, and one to the right of it.

Many saccharides give red-violet pigments with the naphthoresorcinol reagent, but these are insoluble in ether (p. 113).

ESTIMATION OF REDUCING SUGARS IN URINE

This is done by means of Fehling's or Benedict's quantitative method, the micro-modification being most convenient (p. 112).

ABNORMAL METABOLITES IN URINE

(5) **Ketonuria.**—This term denotes the presence of one or all of the compounds, β -hydroxy-butyric acid, acetoacetic acid, and acetone. All are derived from abnormal fat metabolism, and appear in the urine whenever the carbohydrate available is insufficient to participate in the degradation of the fatty acids (p. 319).

There is no simple direct test for β -hydroxy-butyric acid, $\text{CH}_3\cdot\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{COOH}$.

(1) *Iron Test for Acetoacetic Acid.*—Add dilute (1 per cent.) ferric chloride drop by drop to 5 ml. of urine until the buff-coloured precipitate of ferric phosphate, which is given by all specimens of urine, ceases to form. Further addition of the ferric chloride now produces a brown-purple colour if the urine contains more than 0.07 per cent. acetoacetic acid. Urines after administration of salicylates, aspirin, and related drugs, give a violet colour on addition of ferric chloride, which may be mistaken for an acetoacetic acid reaction.

To distinguish, boil a sample for five minutes, and repeat the test. Acetoacetic acid is converted into acetone by boiling and no longer gives a colour with ferric chloride; salicylates are unaffected, and still react.

(2) *Sodium Nitroprusside Test for Acetoacetic Acid and Acetone* (Rothera).—Fill up about 1 in. of a test tube with solid ammonium sulphate. Add 5 ml. of urine, and shake so as to saturate the mixture. Add 2–4 drops of fresh 5 per cent. sodium nitroprusside, and make alkaline with about 10 drops of strong ammonium hydroxide. A deep violet colour develops in a few seconds if the urine contains more than 0.2 per cent. acetoacetic acid, while 0.005 per cent. will give a pink colour in about ten minutes.

A similar colour is given by acetone, but the reaction is not so delicate, although it will reveal 0.01 per cent. in urine.

In dilute urines the colour appears first as a ring at the junction of the liquid and the crystals. The reaction should not be regarded as absolutely negative until the mixture has remained colourless for thirty minutes.

Normal urines rich in organic sulphides (thiols) give an immediate but transient red colour with the reagent. This must not be mistaken for a positive ketone reaction, which is permanent for several hours.

In the earlier form of the test, the ammonium sulphate was not used, but its presence greatly increases the sensitivity of the reaction.

Nitroprusside Reactions in Urine

Reagent.	Creatinine.	Acetone.	Acetoacetic Acid.
Na nitroprusside + NH_4OH Na nitroprusside + NaOH .	no change red (soon fades) ↓	purple red (stable) ↓	purple red (stable) ↓
Acidified with acetic acid .	decolourised	purple	purple
Na nitroprusside + NH_4OH + solid $(\text{NH}_4)_2\text{SO}_4$.	no change	purple	purple

(3) *Diffusion Test for Acetone*.—Place about 1 ml. of Nessler's reagent in a Conway unit or in a small watch-glass resting in a petrie dish. Acidify about 2 ml. of urine with a drop of glacial acetic acid or hydrochloric acid. Carefully pour the urine into the dish around the watch-glass, taking care not to mix the liquids. Replace the cover on the petrie dish. The presence of acetone in the urine is shown by the rapid appearance of a cream-coloured precipitate in the reagent in the watch-glass owing to the diffusion and fixation of the volatile acetone. This reaction is specific for acetone in urine.

(4) *Iodoform Test*.—Addition of 1–5 ml. of 1 per cent. aqueous iodine and 1–2 ml. 20 per cent. NaOH to 10 ml. of a urine containing acetone produces iodoform, CHI_3 , which is recognisable by its smell, and its separation as a pale yellow precipitate with a characteristic microscopic appearance (hexagonal plates and stars). Pyruvic and lactic acid, which are minor constituents of normal urine, give a similar reaction, as also does alcohol, so the test is not reliable for detecting traces of acetone.

Ethyl Alcohol.—The detection of ethyl alcohol in urine may be important in the diagnosis of intoxications. Distil carefully 50–100 ml. of the urine, and apply the iodoform test and the nitro chromic test (p. 109) to 5 ml. samples of the distillate. Alcohol gives a positive reaction with both tests. Acetone, if present, will also come over in the distillate, and give a positive iodoform reaction, but does not give a blue colour with the nitro chromic reagent.

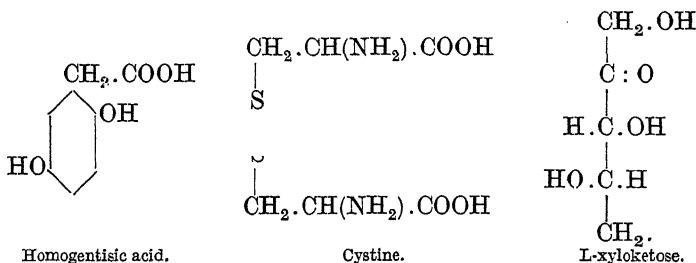
ABNORMAL METABOLITES DUE TO INBORN DEFECTS

Alcaptonuria, characterised by the excretion of homogentisic acid. The urine darkens, owing to oxidative changes, after addition

of alkalis or on undergoing spontaneous ammoniacal fermentation. It reduces alkaline copper reagents in an atypical way, and also gives a transient blue colour on addition of ferric chloride. The homogentisic acid arises from the phenylalanine and tyrosine of the diet.

Cystinuria, characterised by the excretion of cystine, which forms a crystalline deposit of hexagonal plates. The fresh urine has an aromatic smell resembling sweet briar, and gives a black precipitate of lead sulphide on boiling with lead acetate and sodium hydroxide. The daily output is 1-2 gm., being derived from the cysteine and methionine of the dietary protein. Free cystine is not excreted as such by cystinurics, showing the metabolic error is in the breakdown of cystine compounds, which presumably appear as cystine precursors in the urine. The diamines, cadaverine and putrescine often accompany cystine in cystinuria.

Pentosuria, characterised by the excretion of L-xyloketose, which on account of its reducing properties may be mistaken for glucose.



Porphyrinuria, characterised by the excretion of uro- and other porphyrins, which impart a red or brown colour to the urine. The condition appears to be due to a failure of the mechanism for transforming cyclic tetrapyrrols into bile pigments. The accumulation of porphyrins in the blood renders the subject photosensitive to strong light.

Albinism, due to lack of tyrosinase and "dopa" oxidase, which form the natural melanin pigments of skin, hair and retina from tyrosine. The condition is unassociated with any abnormal urinary metabolite. Of these inborn errors, albinism is the most common, cystinuria is moderately rare, and the others are very uncommon. The most dangerous are porphyrinuria, with its hypersensitisation to light, and cystinuria, which tends to the formation of renal calculi.

URINARY SEDIMENTS

According to the modern theory of solutions, dissolved salts are ionised, and consequently the electrolytes in urine are present as :

- I. *Cations* : Na^+ , K^+ , Ca^{++} , Mg^{++} , NH_4^+ .
- II. *Anions* : Cl^- , HPO_4^- , CO_3^- , SO_4^- , $\text{C}_5\text{H}_3\text{N}_4\text{O}_3^-$ (urate), $(\text{COO})_2^-$ (oxalate).

The appearance of a urinary sediment depends on (i.) temperature, (ii.) reaction, (iii.) relative concentration, and (iv.) absence of colloidal anti-precipitants. As secreted, all the urinary constituents are in solution at the temperature of the body, but when urine cools the urates and phosphates tend to separate out if the reaction be favourable. Urine is a better solvent for uric acid than water at the same temperature and pH, a property that appears to be associated with the colloids present in small quantities.

(1) **Carbonates and Phosphates.**—Those of Na, K and NH_4 are freely soluble, and never form urinary precipitates. Those of Ca and Mg are soluble in acid urine ($\text{pH} < 6.5$) but insoluble in neutral or alkaline urine ($\text{pH} > 7.0$), and tend to precipitate when urine cools or becomes alkaline. Such precipitates are easily dissolved by acetic and other weak acids. Phosphatic calculi are among the commonest of the renal and bladder concretions.

(2) **Urates.**—Dibasic alkaline urates, such as $\text{Na}_2\text{C}_5\text{H}_2\text{N}_4\text{O}_3$, are soluble, and never occur as sediments, but the conditions for their existence demand a higher degree of alkali than that present in urine. Monobasic urates, especially ammonium dihydrogen urate, $\text{NH}_4\cdot\text{C}_5\text{H}_3\text{N}_4\text{O}_3$, are sparingly soluble in cold urine, but dissolve on heating or after addition of alkalies. They often appear, along with free uric acid, as an amorphous reddish sediment when urine cools, and are the most common and least important deposit.

(3) **Uric Acid** may accompany urates in very acid urine. Acidify 10 ml. urine with a few drops of strong HCl, and set aside for about twenty-four hours. The uric acid is precipitated as a few dark crystals, heavily pigmented. Remove by pipette and identify microscopically.

(4) **Oxalates** are soluble, with the important exception of calcium oxalate, which separates from acid or alkaline urine in octahedral crystals, and is a common source of calculi.

(5) **Chlorides** and sulphates are sufficiently soluble never to form urinary deposits or concretions.

Urinary Sediments

Acid Urine.	Neutral or Alkaline Urine.
Free uric acid ("cayenne pepper" deposit).	Phosphates and carbonates of Ca and Mg (white, amorphous deposit).
Acid urates of NH_4 , Na or K ("brick dust" deposit).	Ammonium magnesium phosphate (triple phosphate) in ammoniacal urine. (Large, prismatic crystals.) Colourless.
Calcium oxalate, usually in very small amounts. ("Envelope" crystals.)	Ammonium urate.
	Calcium oxalate.

GENERAL REFERENCES

- BEAUMONT, G. E., and E. C. DODDS (1939), "Recent Advances in Medicine." 9th Ed. London.
- COLE, S. W. (1933), "Practical Physiological Chemistry," 9th Ed. Cambridge.
- DUKES, C. (1939), "Clinical Pathology of the Urine." London.
- HARRISON, G. A. (1937), "Chemical Methods in Clinical Medicine," 2nd Ed. London.
- HAWK, P. B., and O. BERGEIM (1937), "Practical Physiological Chemistry," 11th Ed. London.
- PETERS, J. P., and D. D. VAN SLYKE (1932), "Quantative Clinical Chemistry," Vol. II. London.

CHAPTER XXIV

HORMONES

“Except during the nine months before he draws his first breath, no man manages his affairs as well as a tree does.”

G. B. SHAW.

Hormones, or autacoids, are autogenous compounds manufactured and employed for the chemical co-ordination of the organism. They are specific chemical reactants produced by a tissue, and have the characteristic of exerting an effect of functional value on tissues or organs.

Classification.—The following classification is that proposed by Huxley (1935), with the exception that his use of the term *activator* to include all these chemical regulators has been changed in favour of the term *autacoid* (αὐτός, self; ἀκος, remedy), originally suggested by Schafer.

They are termed (i.) *internal secretions*, or endocrines, on account of the fact that many are sent directly into the blood stream from the tissue that produces them; and (ii.) *hormones*, from the fact that many but not all are physiological stimulants (ἐρμάω, I excite).

(1) **Local autacoids**, affecting the same cell or tissue in which they are produced.

(i.) *Intracellular hormones*.

(ii.) *Regional hormones*, which are responsible for the pre-determination of specific regions in the embryo, such as the limb-disc, the eye-rudiment and the chick pronephros.

(2) **Diffusion autacoids**, transported by diffusion through the tissues.

(i.) *Growth hormones of plants*, such as the auxins.

(ii.) *Evocators and organisers* of the vertebrate embryo.

(iii.) *Neurocrines*, or chemical transmitters of the nerve impulse.
Cholinergic and adrenergic factors.

(3) **Circulating hormones**, which are distributed by the blood stream or lymph to all parts of the organism. These are represented by the endocrine secretions of the ductless glands.

(4) **Para-hormones**.—By-products of normal and pathological metabolism. Carbon dioxide, the regulator of the respiratory centre. Histamine, the regulator of capillary blood volume.

General Characters of Hormones.—(1) *High Potency*.—Autacoids

resemble many of the alkaloids and the vitamins in being effective in very small dosages.

(2) *Tolerability*.—Being of autogenic origin, the autacoids do not evoke anaphylactic responses or immunity in the organism.

(3) *Relatively Low Molecular Weight and Simple Structure*.—This is true of adrenaline and thyroxine, but does not apply to the hormoproteins.

(4) *Thermal Stability*.—Unlike enzymes, autacoids are not destroyed by boiling for short periods in neutral solutions. Decomposition may occur in presence of strong alkalies, acids, or oxidisers.

(5) *Biological Lability*.—Many autacoids are readily destroyed after they have entered the circulation, and, in consequence, have a temporary and local effect. Others, such as oestrone, are excreted in the urine after slight modification.

(6) With few exceptions, autacoids are destroyed in the alimentary tract, and are ineffective when administered orally, unless in enormous dosage.

The existence of an autacoid is established in six different ways :—

(i.) Histological recognition of endocrine tissue. This led Schafer to conclude that an internal secretion was located in the islet tissue of the pancreas, and he suggested the name *insuline* many years before the actual autacoid was discovered.

(ii.) Pathological conditions associated with changes in endocrine tissue. Examples are Addison's disease (suprarenals), Graves's disease (thyroid), Fröhlich's syndrome (pituitary).

(iii.) Physiological response to administration of endocrine extracts.

(iv.) Isolation and identification of the active principle.

(v.) Production of characteristic pathological conditions by removal of endocrine tissue.

(vi.) Adequate compensation for endocrine deficiency by administration of autacoid, or gland extract, or by gland implantation.

Significance and Mode of Action.—Langdon Brown has suggested that the animal autacoids are the survival of the primitive chemical apparatus which regulated the organism before the evolution of a central nervous system, which, when it arose, became allied to the pre-existing endocrine system in two ways. The sympathetic mechanism became associated with the adrenal, thyroid and pituitary glands; the parasympathetic mechanism became associated with the insular tissue of the pancreas and with the choline bases.

The circulating hormones have been evolved in association with the appearance of highly specialised tissues, which are represented in all vertebrates by the adrenal, thyroid, pituitary and sex glands.

The parathyroids and thymus first appear in the amphibia, while the corpus luteum and placenta are restricted to placental mammals.

Animal Hormones

Source.	Hormone.	Action.
(1) Adrenal cortex .	Cortin. (Corticosterone.)	Metabolic.
(2) Adrenal medulla .	Adrenosterone. Adrenaline.	Sodium control. Androgenic. Sympathomimetic. Gluco-kinetic.
(3) Thyroid . . .	Thyroxine.	Metabolic.
(4) Parathyroid . . .	Parathyrin.	Calcio-kinetic.
(5) Pancreas . . .	Kallikrein. Insulin.	Vaso-dilator. Gluco-kinetic.
(6) Ovary : . . .	Œstradiol.	Œstrogenic.
Follicular hormones	Œstrone. Œstriol. Equilin. Equilenin.	Œstrus control.
Luteal hormone	Progesterone.	Endometrium control. Placental development.
(7) Testicle. . . .	Testosterone. Androsterone. Dehydroandrosterone. Diffusing factor.	Androgenic.
(8) Anterior pituitary	Growth factor. Œstrogenic factor. Luteinising factor. Prolactin. Thyrotropic principle. Adrenotropic principle. Pancreotropic principle.	Skeletal development. Follicle stimulation. Luteal stimulation. Mammary growth. Thyroid stimulation. Adrenal stimulation. Insular tissue stimulation.
	Metabolic principle.	General metabolic stimulation.
(9) Posterior pituitary	Ketogenic principle. Glycotropic principle. Anti-diuretin. Vaso-pressin. Oxytocin.	Lipo-kinetic. Anti-insulin. Diuresis control. Vaso-constrictor. Muscle contraction.
(10) Gastric mucosa .	Gastrin.	Gastric secretion.
(11) Duodenal mucosa .	Secretin. Cholecystokinin.	Pancreatic secretion. Gall-bladder control.
(12) Spleen	Acetyl choline.	Vaso-dilator.
(13) Placenta . . .	Œstriol.	Œstrogenic.

ADRENAL HORMONES

A. Adrenal Cortex . . .

The adrenal gland is composed of two structures, independent in history and physiological function. The cortex is of mesoblastic origin, and arises from the coelomic epithelium on either side of

the root of the mesentery; the medulla arises from the primitive nerve tissue beside the posterior root ganglia, which subsequently differentiates into the sympathetic nervous system. Unlike the medulla, the adrenal cortex is necessary for life, and for this reason removal of the entire gland is fatal in animals not equipped with accessory adrenal tissue.

(1) **Cortin, Corticosterone.**—By alcoholic extraction and fractionation, active preparations of adrenal cortex have been obtained by several workers, and are represented by "cortin" (Hartmann, 1928), "interrenin" (Goldzieher, 1928), "interrenalin," "eucortin" (Swingle and Pfiffner, 1930), "interrenalin" (Rogoff, 1931). In 1934, Kendall and his colleagues obtained the hormone as a crystalline mixture of closely related steroids. Comparable results were reported, in 1936, by Reichstein, who has applied the name *corticosterone* to the most active of his compounds, $C_{21}H_{30}O_4$, to denote that it is a steroid ketone. Many related compounds of varying potency occur in cortical extracts.

Effects of Cortical Deficiency.—(1) *Metabolic.*—Complete removal of the cortical tissue from an animal results in death within a week. There is rapid loss in weight, general muscular weakness, fall in temperature and reduction in basal metabolism by about 25 per cent. The syndrome is seen in a less acute form in partial removal of the cortex, and in pathological dysfunction, as in Addison's disease and in vitamin B_2 deficiency.

The cortex controls phosphorylation, and when this is disturbed the absorption of hexoses by the intestine and renal tubules is repressed, with consequent disorder of sugar traffic.

(2) *Sodium Regulation.*—Cortical deficiency is characterised by an increase in the urinary output of chloride, and a resulting fall in sodium, potassium and their ionic partners, chloride and bicarbonate, in the plasma. Conversely, administration of sufficient NaCl to keep the plasma level normal has a beneficial effect in the treatment of both experimental and Addisonian cortical deficiency.

(3) *Androgenic.*—Dysfunction of the cortex owing to tumour formation (hypernephromata) in early childhood is associated with precocious sexual development in girls, but not in boys. Dysfunction in adult females leads to the appearance of the secondary sexual characters of the male. From this it is inferred that an androgenic or masculine autacoid is being elaborated in the gland, and one such factor, *adrenosterone*, $C_{18}H_{24}O_3$, has been isolated from the normal gland. Gordon Holmes has found that removal of a cortical tumour resulted in the restoration of the secondary female sexual characters.

The adrenal cortex is very rich in steroids and in ascorbic acid, and is either a factory or a storage depot for these reactants. The metabolic disturbances due to cortical deficiency may be associated with lack of ascorbic acid, and there is evidence that this vitamin is of therapeutic value in the treatment of Addison's disease (Sclare, 1937). The sexual disturbances associated with cortical dysfunction in the female originate probably from abnormal sterol metabolism, as a result of which the tissue either produces an androgen or fails to destroy one.

B. Adrenal Medulla

(2) **Adrenaline**, or epinephrine, constitutes about 0.1 per cent. of fresh medullary tissue, and is present to a much lesser extent (0.02 per cent.) in the cortex. It is obtained from alcoholic or aqueous extracts of the gland, and is precipitable by ammonium hydroxide. The pure base is a crystalline solid, moderately soluble in water, and forming stable salts with strong acids. Structurally it is a catechol derivative of a methylated amino alcohol, and has been synthesised.

Effects of Adrenaline Injection.—Adrenaline is a sympathomimetic amine, and produces effects similar to the stimulation of structures innervated by the sympathetic system, the sweat-glands being a notable exception.

(1) *Vaso-constriction.*—Splanchnic and cutaneous arterioles are constricted, the arterioles of the skeletal muscles are unaffected or may be dilated. This leads to a redistribution of blood in the organism.

(2) *Cardiac Stimulation.*—The rate and force of the heart-beat are increased, the effect suggesting a heightened sensitivity to the Ca^{++} ion. The coronary arteries are dilated.

(3) *Glycogenolysis and Increase in Lactic Acid Content of Blood.*—This is accompanied by a corresponding breakdown of muscle glycogen. The excess lactic acid is resynthesised to glycogen in the liver, and in turn serves to raise the level of blood sugar. Thus, adrenaline injection causes a mobilisation of carbohydrate in the blood, and may be described as a *glucokinetic hormone*.

(4) *Miscellaneous Muscular Responses.*—Dilatation of the pupil, retraction of the upper eyelid, and protrusion of the eyeball; relaxation of the bronchi, relaxation of the detrusor, and contraction of the sphincters in the bladder. Inhibition of movement, and contraction of the sphincters of the intestine.

In man, the therapeutic subcutaneous dose is 10 minims of a 0.1 per cent. solution, which represents about 0.003 mg., per kg. body weight.

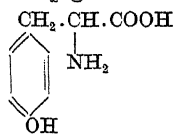
Effects of Adrenaline Administration.—Injected subcutaneously the absorption of adrenaline is retarded owing to the local vasoconstriction it produces, and so it is administered intramuscularly or intravenously. The general cardio-vascular response depends on the integrity of the vagal innervation of the heart. If this be abolished, by section or by atropine, the response to adrenaline is a rapid, temporary rise in blood pressure, accompanied by an increase in the frequency and force of the heart beat. The effect subsides in a few minutes owing to destruction of the hormone. Where the vagal innervation is intact, moderate doses of adrenaline (0.1–0.5 mg.) cause a rise in blood pressure, accompanied by slowing of the heart, owing to vagal inhibition evoked by an aortic pressure reflex. Large doses of adrenaline (1.0–1.5 mg.) causes a 20 per cent. increase in the basal metabolic rate, and a two-fold increase in the cardiac output, the stimulus from the hormone being sufficient to overcome vagal inhibition. The cardiac effect may persist for over an hour, and may be accompanied by disturbances of rhythm, terminating in heart failure.

Significance of Adrenaline.—By means of cross-circulation experiments in which the blood of an intact animal was conveyed to the jugular vein of an adrenalectomised animal, Tournade and Heymans obtained evidence to show that adrenaline is continually being secreted in all conditions of bodily activity, reaching maximal values in (i.) physical stress, (ii.) exposure to cold, (iii.) fall in arterial blood pressure, (iv.) asphyxia, (v.) hypoglycæmia, (vi.) central anaesthesia, (vii.) sudden or painful stimuli, and (viii.) emotional disturbance. Cannon for many years has maintained that adrenaline is the hormone concerned in the expression of terror, rage, panic and pain. By accepting the psychological implication of this theory, adrenaline appears as the chief effector agent employed by the unconscious self for the emergency defence of the organism.

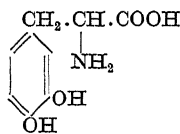
The adrenaline output is controlled by a higher nervous centre, which responds to (i.) stimuli from increase in blood pressure, these stimuli being transmitted from the aorta and carotid sinus, and serve in the maintenance of the circulation; (ii.) chemical stimuli from the sugar in the blood, which when it falls below its usual level, evokes a compensatory release of stored carbohydrate; (iii.) hormone stimuli from the adrenotropic factor of the anterior pituitary; and (iv.) thermal stimuli, when the temperature of the external or internal environment falls below a critical value. By its response to each of these type of stimuli, the adrenal gland sets in action the mechanism appropriate for meeting each specific demand. Emotional disturbances also may evoke liberation of adrenaline. The efferent impulses to the gland travel in the splanchnic nerves, and when these are cut the emergency secretion of adrenaline ceases.

Chemistry of Adrenaline.—In 1856, Vulpian observed that the adrenal medulla was coloured green by ferric chloride, and red by oxidisers such as iodine or chromate; and the term *cromaffin* was applied to the tissue. Extracts of the medulla showed similar chromogenic properties, which facilitated the isolation of the crystalline hormone, in 1901, by Takamine and by Aldrich, who showed that the molecular formula was $C_9H_{13}O_3N$.

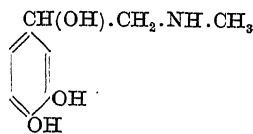
The green colour reaction with ferric salts indicated the presence of a catechol, or *o*-dihydroxy benzene nucleus, and this was confirmed by the production of protocatechuic acid (3:4-dihydroxy benzoic acid) on fusion of adrenaline with KOH. Von Fürth showed that a methylamino and a secondary alcohol group were present in the form of a side-chain, $-\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{NH}\cdot\text{CH}_3$, from which the carboxyl group of the protocatechuic acid was derived. Thus, the formula of adrenaline is 3:4-dihydroxyphenyl-ethanolmethylamine, and it is related both to tyrosine and to the tyrosine metabolite, dihydroxyphenyl alanine (dopa). Adrenaline has been synthesised independently by Stolz (1904) and by Dakin, starting from catechol. Like the other dihydroxy phenols, adrenaline is unstable in alkaline solution, and readily oxidised to a red pigment.



Tyrosine.



"Dopa."



Adrenaline.

Assay of Adrenaline.—The hormone value of preparations may be estimated (i.) *colorimetrically*, by comparison of the blue colour it yields with Folin's phosphotungstate reagent; (ii.) *biologically*, from the inhibitory effect on the contraction of a strip of involuntary muscle suspended in saline, the accelerating effect on an isolated heart, the dilating effect on a denervated iris, and the vaso-pressor response in an animal.

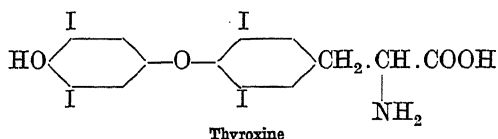
Adrenaline is optically active owing to an asymmetric carbon atom in the side-chain. The natural form is the (+)-isomer, and is much more active than the (−)-isomer.

THYROID HORMONE

The thyroid gland arises as an outgrowth of the pharyngeal floor in the embryo. In the human adult it weighs 20–25 gm., and is unique among the tissues because of its high content of iodine, the average amount being 15 mg., at least half of which can be extracted in the form of the hormone thyroxine.

L-Thyroxine was isolated by Kendall, in 1914, and identified by Harington and Barger, who subsequently effected its synthesis (1927). The details of this achievement are summarised by Barger (1930).

Thyroxine is derived from two molecules of di-iodo-phenol, and contains an alanine side-chain, thus being related to tyrosine, its precursor in the diet, and to iodogorgic acid. Owing to the asymmetric carbon atom in the side-chain, two forms of thyroxine are known, the natural hormone being the L (–)-isomer, derived from L-tyrosine.



There is reason to believe that the actual hormone is a compound of thyroxine, since preparations of gland protein have been obtained that are more effective than pure thyroxine, and act more rapidly. Also, thyroxine has been isolated from the thyroid as a peptide component. The residual iodine of the gland is present as the physiologically inert di-iodo-tyrosine.

Effects of Thyroid and Thyroxine Administration.—Thyroid preparations are exceptional among the autacoids in that they are equally effective when administered by the mouth, from which it is concluded that the gland originally sent its secretion into the primitive alimentary tract.

(1) *Stimulation of Metabolism.*—One mg. of thyroxine can raise the basal metabolic rate of the adult by 2–3 per cent. Larger doses cause a proportionate increase and a prolonged effect. This stimulation is accompanied by a loss in weight, owing to depletion of the fat reserves, and hypertrophy of the active organs, heart, kidney, suprarenal, and liver.

(2) *Mobilisation of glucose*, owing to increased breakdown of hepatic and cardiac glycogen. The blood sugar level is raised, and hyperglycæmia and glycosuria may result.

(3) *Decalcification of bone.* The effect differs from that of the parathyroid hormone in that the plasma calcium level is not raised and the transferred calcium is excreted by the intestine as well as in the urine.

(4) *Stimulation of metamorphosis* in amphibia, including the tadpole.

(5) *Species desensitisation to methyl cyanide* (aceto-nitrile). A method for assaying thyroid preparations is based on the increased tolerance to methyl cyanide that they confer on mice.

Hypothyroidism.—A. *Acute.*—Thyroidectomy, uncomplicated by involvement of the parathyroids, in young animals causes cretinoid conditions to develop, including retardation of skeletal growth, hardening of skin and loss of hair, retarded sexual development and absence of secondary sexual characters, and mental dullness and apathy. In man, the condition of *cachexia strumipriva* (κακός, ill; ξῆς, habit; *struma*, goitre; *privus*, deprivation), or operative myxœdema, develops in a week, as shown by: fall in basal metabolic rate, usually by about 25 per cent.; slowing of the heart beat to about 50 p.min.; subnormal temperature; cutaneous changes, loss of hair from scalp and outer third of eyebrows, subcutaneous deposition of myxomatous tissue; disturbance of mental activity and sexual functions.

B. *Chronic.*—Non-development or atrophy of the gland.

(1) Congenital maldevelopment causes **cretinism**, the signs of which do not appear until about six months after birth, due partly to the autacoids supplied in the mother's milk and partly to the reserve in the child. The signs and symptoms of cretinism are similar to those of hypothyroidism or myxœdema.

(2) Simple goitre accompanied by hypothyroidism may arise when there is a deficiency of iodine in the diet. This is due usually to a faulty environment. A compensational hypertrophy of the gland endeavours unsuccessfully to manufacture sufficient autacoid from inadequate material.

(3) Atrophy of the thyroid may occur spontaneously in adults, especially women, after middle age.

All these conditions are specifically relieved by appropriate thyroid administration. One mg. of iodine *per diem*, supplied in any inorganic form, is sufficient to cure simple goitre and prevent its reappearance.

Hyperthyroidism.—Increased secretion of the hormone may occur without obvious signs of gland enlargement, the result being: (i.) increased basal metabolism, (ii.) mild pyrexia, (iii.) pigmentation of the skin, (iv.) nervous restlessness, (v.) mild exophthalmos, (vi.) cardiac and vascular disturbance.

Hyperthyroidism is a feature of Graves's disease (Basedow's disease, or exophthalmic goitre), the pathology of which is still obscure. In Graves's disease the basal metabolic rate may be 50 per cent. above normal in an average case, and up to 100 per cent. above normal in a severe case.

Metathyroidism.—Production of abnormal secretions of the gland may complicate many varieties of Graves's disease and toxic adenomata of the gland. The condition is sometimes termed "thyrotoxicosis," "toxic goitre," and "dysthyroidism." The chief

symptoms are due to hypersensitivity of the sympathetic system. Metathyroidism is distinguished from hyperthyroidisms by being benefited by iodine administration, which suggests that it is due to liberation of an immature or defective autacoid. Harington, however, can find no evidence of an abnormal hormone.

The most obvious histological effect of the iodine therapy is the restoration of the colloidal appearance to the gland structure.

Regulation of Thyroxine.—The thyroid gland is supplied by the middle cervical sympathetic ganglion, but section of the entire nerve supply does not lead to hypothyroid states, from which it is inferred that the gland has a metabolic activity of its own, and continually elaborates, stores and secretes its hormone independently of the autonomic nervous system. Apart from the obvious limiting factors, namely, the supply of iodine and tyrosine or phenyl alanine, the gland responds to (i.) hormone control by the thyrotropic factor of the anterior pituitary, and (ii.) thermal stimuli, which, perhaps, act through the thermotaxic centre in the hypothalamus, and reach the gland by the sympathetic nerves.

Cramer has shown that exposure to cold evokes a protective secretion by the thyroid as well as by the adrenal gland.

Therapeutic Applications of Thyroxine.—In addition to their specific use in the hypothyroid states, thyroid preparations are used popularly in the treatment of obesity. Given to a normal subject, small doses lead to a disappearance of storage lipides, provided the diet is kept sufficiently low in fat and carbohydrate to compel the subject to draw on his own reserves. Overdosage leads to alimentary disturbances, tachycardia and loss in weight. Other drugs, notably di-nitro phenol and di-nitro *o*-cresol, have a similar effect in increasing general metabolism, but they are dangerous remedies in comparison with the natural hormone. Unlike thyroxine, they display a catalytic effect in promoting respiration in tissue slices (Dodds).

Significance of Thyroxine.—The iodine content of the thyroid gland varies greatly with the environment and the diet. The dried preparations of ox, sheep or pig gland, used therapeutically, are standardised by admixture with sufficient lactose to give a powder containing 0.1 per cent. iodine in the form of thyroxine. The iodine content of human and other animal thyroids ranges from 0.1 to 0.5 per cent. of the dry weight, but may exceed 1.0 per cent. in animals living on marine foodstuffs rich in the halogen, such as Orkney sheep, which feed on kelp seaweed. When the iodine content falls below 0.1 per cent. of dry weight, the gland enlarges and assumes the goitrous form.

Thyroxine has only about half the activity of a thyroid prepara-

tion containing the same amount of iodine, from which it appears either that another and much more potent autacoid is present, or that thyroxine is merely a constituent of the actual autacoid.

Furthermore, thyroxine differs from adrenaline in that the response to its administration is very slow and sustained. Adrenaline reacts within a minute; thyroxine requires two to four days. This suggests that thyroxine requires physiological modification before becoming available for metabolic catalysis.

The daily output of thyroxine in the human adult is believed to be of the order of 0.5–1.0 mg. *per diem*, which is the amount sufficient to maintain normal health in the myxoedematous subject.

The iodine thus metabolised is not lost to the organism, but is in part re-synthesised to thyroxine. The urinary output of iodine ranges from about 0.03–0.173 mg. *per diem*, depending on the diet, and von Fellenberg has computed that the minimal requirement of the human subject is met by the daily provision of 0.05 mg. I_2 in any soluble inorganic form.

From its effect in the myxoedematous condition, it is estimated that each milligram of thyroxine utilised increases the energy output by about 1,000 kilocalories, an amount equivalent to that obtained by the oxidation of 250 gm. of carbohydrate.

Artificial metabolic stimulants, such as di-nitrophenol, are also effective in raising the metabolic rate to the normal level when administered to myxoedematous subjects, but fail to benefit the other associated disturbances, which is additional proof that the thyroid gland action controls other processes in addition to its calorogenic action.

Thyroxine added to tissue preparations has little or no effect on tissue respiration, though it is claimed that tissues from animals suffering from hyperthyroidism display an increase in metabolic activity, including glycolysis and lactate formation.

PARATHYROID HORMONE

The parathyroids are the smallest endocrine organs known in the animal body, and are represented by four structures situated at the back of the thyroid gland. Before their existence was recognised, they were removed with the thyroid tissue during experimental thyroidectomy, and the effects of the operation included both hypothyroid and hypoparathyroid phenomena.

Parathyrin, or parathormone (Collip), is obtained by extracting fresh parathyroid tissue with 5 per cent. HCl, and precipitating the proteins. The autacoid is freely soluble in water and in alcohol up

to 92 per cent. strength. Subcutaneous or intravenous injection causes :—

(1) *Mobilisation of Blood Calcium*.—The Ca level is raised from the normal value of about 10 mg. per 100 ml. to a value depending on the number of autacoid units given.

The unit is one-hundredth of the amount required to cause a 5 mg. Ca increase per 100 ml. in the blood serum of a 20 kg. dog, fifteen hours after injection.

(2) *Abolition of Tetany*.—All forms of tetany due to hypocalcæmia are relieved, including those directly due to parathyroid removal. This includes infantile, post-operative, and parathyroid tetany, as well as that due to alkalosis.

(3) *Hypophosphatæmia*.—The rise in blood calcium is associated with a fall in blood phosphate.

Hyperparathyroidism.—A. *Acute*.—Collip has shown that over-dosage of parathyrin causes severe hypercalcæmia in which the Ca level may rise above 20 mg. per 100 ml. This is accompanied by (i.) depression of motor excitability ; (ii.) anorexia, drowsiness, and coma ; (iii.) diarrhœa ; (iv.) decrease in blood volume and increase in viscosity.

Death is due to failure of the heart, and is followed rapidly by intravascular clotting.

B. *Chronic*.—Clinical hyperparathyroidism has been recognised by Barr and other workers. The features are : (i.) hypercalcæmia ; (ii.) negative calcium balance with hyperexcretion of calcium in the urine ; (iii.) bone rarefaction ; (iv.) muscular weakness and hypotonia.

Hypoparathyroidism.—A. *Acute*.—Complete parathyroidectomy in the dog is followed by : (i.) hypocalcæmia, the serum Ca level falling by 50 per cent. to about 6 mg. per 100 ml. ; (ii.) hyper-excitability of the peripheral nerves to electrical and mechanical stimuli, muscular twitching ; (iii.) tetanic spasm, due to central stimulation in the brain stem. These increase in violence, with alternate phases of exhaustion, until death results from asphyxia owing to respiratory spasm, or cardiac failure.

Acute hypocalcæmia may be treated by oral administration of large doses of soluble calcium salts, by saline injections, and, specifically, by injections of parathyrin. After about six weeks the organism becomes adapted to life at a lower level of serum calcium.

B. *Chronic*.—Idiopathic hypoparathyroidism and some forms of infantilism exemplify the chronic condition. Infantile tetany, which is always associated with rickets, is not regarded as being a parathyroid disorder.

Hypoparathyroidism, as far as it can be regarded as a clinical entity, displays : (i.) hypocalcaemia ; (ii.) hyperphosphataemia ; (iii.) abnormalities of ossification ; (iv.) potential tetany. Probably the age of onset of the disease determines the nature of the symptoms.

Significance of Parathyrin.—This is a calcio-kinetic hormone, regulating the level and distribution of Ca between the skeletal tissues and the circulating fluids.

Parathyrin output is controlled (i.) by a parathyrotropic factor of the anterior pituitary, and (ii.) indirectly, by the Ca-ion concentration of the plasma. About half the plasma calcium is in a colloidal state, probably as a protein carbamate, and the hormone may be concerned in maintaining the equilibrium between available ionic and non-available organic forms of the metal.

Tetany.—Clinical tetany is characterised by the tendency of muscles to undergo spasmodic contraction, those commonly affected being the muscles of the forearm and hands, the larynx, and, less frequently, the lower limbs. The condition is associated with a fall in the plasma calcium level below 7 mg. per 100 ml., and an accompanying rise in the phosphate level to above 5 mg. per 100 ml. Tetany is recognised clinically by :

(i.) *The Chvostek Sign.*—Twitching of the upper lip and side of the face elicited by tapping the facial nerve in front of the ear.

(ii.) *The Trousseau Sign.*—Carpal spasm elicited by pressure on the upper arm.

(iii.) *Laryngismus Stridulus.*—Closure of the glottis owing to sudden spasm of the laryngeal muscles. Inspiration ceases until the muscles relax, when the air re-enters the lungs with a characteristic sound.

Latent tetany can be recognised by the increased excitability of the motor nerves to a constant, low voltage electrical stimulus ; the cathodal opening response being evoked by a current of 0.6–3 milliamperes, the normal requirement being about 6.0 milliamperes.

PANCREATIC HORMONES

History.—In 1889, Mehring and Minkowski found that extirpation of the pancreas in dogs was followed by severe glycosuria and fatal coma, the condition being indistinguishable from diabetes mellitus. Ligation of the gland duct, although causing digestive disturbances from lack of pancreatic juice, did not evoke this artificial diabetes, from which it was concluded that a hormone or internal secretion was still being manufactured.

In 1916, Schafer suggested that this hormone was manufactured

in the insular tissue of the gland, and suggested the name *insuline*. Previous and later attempts to extract this hormone had been inconclusive, although Zuelzer (1907) and Scott (1911) undoubtedly obtained active preparations, until, in 1920, Banting and Best devised a technique for separating the autacid from the destructive influence of the pancreatic enzymes.

By continuous injection of islet extracts, a depancreatized dog was kept alive for seventy days in 1922. Less than two years later, insulin was in use all over the world in the successful treatment of diabetes.

Preparation of Insulin.—(a) *Alcohol Fractionation* (Collip, 1923).—Fresh minced pancreas is extracted with aqueous alcohol, the extract is concentrated *in vacuo* until most of the fat has separated out, alcohol to 80 per cent. is added to precipitate the proteins, and then insulin in the filtrate is precipitated by raising the alcohol concentration to 92 per cent. By using alcohol made alkaline with NaHCO_3 for preliminary extraction, and subsequently acidifying, the yield can be increased fivefold.

(b) *Picric Acid Precipitation* (Dickens and Dodds, 1924).—The fresh gland is ground up with solid picric acid, extracted with acetone, and the insulin picrate recovered after distillation of the solvent.

Crystalline insulin, $\text{C}_{45}\text{H}_{69}\text{O}_{14}\text{N}_{11}\text{S} \cdot 3\text{H}_2\text{O}$, has been obtained by various methods, including the addition of pyridine to insulin solutions at pH 5.6. The potency of the crystalline hormone is very little greater than that of some of the amorphous preparations, namely, 23–26 international units per mg. Chemically, insulin belongs to the peptides, which may explain its destruction in the alimentary tract, when given by the mouth.

Effects of Insulin Injection.—(1) *Hypoglycæmia*.—The level of the blood sugar falls rapidly within thirty minutes of the injection; and, if the dose be sufficiently large, and the carbohydrate reserves depleted by starvation, the condition of hypoglycæmia sets in, the blood sugar level having fallen below 0.04 per cent.

The condition in the rabbit is marked by signs of extreme hunger, violent convulsions, coma, and death from respiratory failure. Rigor mortis follows immediately. In the human subject, hypoglycæmia is accompanied by hunger pangs, exhaustion, tremor, vaso-motor disturbances, sweating, delirium, and coma.

All these hypoglycæmic phenomena may be abolished in a few minutes by intravenous injection of glucose, or less rapidly, by oral administration. Fructose and mannose are not so effective; galactose is almost without action. Injection of the other glucokinetic autacoids, namely, adrenaline and pituitrin, may cause a

rise in blood sugar sufficient to compensate for the effects of the insulin.

- 1 Insulin injection is followed by an increased metabolism and a rise in the respiratory quotient, showing that the utilisation of sugar has been stimulated.

The hypoglycaemia, however, is greater than can be accounted for by the immediate combustion of glucose, and two other factors are believed to act in lowering the blood sugar level: (i.) increased storage of carbohydrate, and (ii.) decreased sugar formation from the glucogenic amino acids. Accompanying the rise in carbohydrate metabolism, is an immediate inhibition of ketone formation from perverted fat metabolism.

(2) *Glycogenesis*.—When a diabetic animal is given insulin and more than sufficient sugar to compensate for the insulin hypoglycaemia, it is found that some of the excess of the saccharide has been stored up in the liver and muscles as glycogen. This glycogenic effect is not seen usually after insulin injection because the glycogen stores are rapidly depleted in an endeavour to maintain a normal sugar level in the blood.

Muscle glycogen represents a much more stable location of the polymer than hepatic glycogen. Severe hypoglycaemia ultimately results in an almost complete withdrawal of liver glycogen to meet the aggravated metabolic demands, whereas even a fatal hypoglycaemia may leave an abundant reserve of muscle glycogen.

(3) *Hypophosphatemia* follows insulin injection, probably owing to a diversion of serum phosphate to form labile hexose phosphate preparatory to sugar utilisation.

The general effects of insulin are expressed by a hypoglycaemia attributable to (i.) increased sugar oxidation, (ii.) increased sugar storage, chiefly in the muscles, and (iii.) decreased sugar formation from non-carbohydrate sources.

The Insulin Unit.—This was defined originally as the amount required to evoke hypoglycaemic convulsions in a fasting rabbit of 2 kg. weight. Wide variations in animal sensitivity have led to the abandonment of this unit in favour of one based on the use of a dry, soluble insulin hydrochloride preparation kept in the National Institute for Medical Research, in London. One insulin unit equals 0.125 mg. of this material.

Insulin forms a salt with protamines that is less soluble and more prolonged in action than the free hormone, and this therapeutic effect may be extended by traces of zinc (p. 23).

Regulation of Output.—The hormone is continually being secreted to meet demands of carbohydrate metabolism, and is present in the venous blood leaving the pancreas. Secretion is evoked

(i.) *chemically*, by a rise in the sugar content of the blood, and (ii.) *neurally*, by the vagi which innervate the insular tissue, and which on stimulation bring about a fall in blood sugar. The sugar factor in the secretion of insulin has led some workers to attribute diabetes mellitus to failure of the gland owing to prolonged exhaustion by a diet excessively rich in carbohydrate. This notion in a less explicit form inspired the earlier methods of treatment of the disease by complete exclusion of all starches and sugars from the diet, so that the gland might recuperate. The discovery of insulin, however, has made it possible to utilise carbohydrate foodstuffs, and many types of dietary are now available for the diabetic subject. The occasional appearance of an insulin-resistant type of diabetes is attributed to dysfunction involving other gluco-kinetic hormones, notably those of the anterior pituitary.

Significance of Insulin.—The hormone is a crystallisable peptide of m.w. 35,000, and contains seven known amino acids: arginine, histidine, lysine, leucine, tyrosine, cysteine and glutamic acid. Because of its low diffusibility it might be expected to interact with the glucose in the blood rather than accompany its substrate into the tissues, but up to the present time no glycolytic property has been ascribed to insulin when mixed with glucose in aqueous solutions or when added directly to blood. It is possible that insulin activates glucose in some way not revealed by the ordinary reduction or polarimetric tests, but it is much more probable that some unidentified agent also participates in the reaction. The existence of a non-pancreatic factor was established when Houssay, Lucke and other workers (1931–37) showed that removal of the pituitary gland from diabetic animals resulted in a cessation both of glycosuria and ketonuria, thus rendering untenable the theory that diabetes is of purely pancreatic origin. This effect of the pituitary is provisionally explained by endowing it with two hormones; (i.) a *diabetogenic* factor, which antagonises insulin, and, if present in excess, leads to a hyperglycæmia of the insulin-resistant type, and (ii.) a *ketogenic* factor, which promotes the degradation of fatty acids, and, possibly, their transformation into glucose or glycogen.

GYNÆCOGENS: FEMALE SEX HORMONES

In order that the genetically determined vertebrate sex may develop to maturity and function, hormones elaborated by the germinal glands are necessary. These are sometimes termed the secondary sexual hormones to distinguish them from the primary sexual autacoids responsible for the sex of the embryo, which at the present time is beyond the scope of experimental control. The

various aphrodisiacs employed by the human race have a story that goes back to the fruit tree of Eden, and includes the fantastic pharmacologies of the East as well as the potions of the Middle Ages. It is now established that the sex hormones, both male and female, are lipid compounds related to the sterols, and are only absorbed with difficulty from the alimentary tract, which may explain the erratic results obtained by empirical therapy.

The experimental foundation of modern knowledge dates from 1912, when Nussbaum showed that the male sex characters of the frog are controlled by a testicular hormone, and that whenever specific structures are associated with animal sex their growth is dependent on chemical factors. Because of the greater complexity of the female organism, two types of hormone are required: (i.) an *œstrogen*, controlling the uterine cycle, and (ii.) a *progesterone*, which prepares the uterus for the implantation of the fertilized ovum.

Progress in the study of the human hormones was delayed, partly through ignorance of the exact nature of the menstrual cycle, and partly because of the lack of a satisfactory test for measuring the activity of the hormone preparations. In 1923, Allen and Doisy obtained a lipid extract from ovaries which, when injected into castrated rodents, was able to re-establish the reproductive or œstrus cycle, and obviously contained the long-sought œstrogen. The same workers also adapted a "vaginal-smear" test, whereby it was possible histologically to detect the onset of œstrous from changes in the vaginal epithelium.

In 1927, Aschheim and Zondek found that an œstrogen was excreted in large quantities in the urine of pregnant animals, thus providing a source of material for purification and identification. Since then, five closely related œstrogens have been isolated from the ovary, the urine of pregnancy, and from the placenta; a progesterone has been isolated from the corpus luteum, and four *androgens*, or male sex hormones have been isolated from various sources. All are derived from a parent *cyclo-pentano phenanthrene* nucleus, and represent the simplest steroids found in the animal organism (p. 181).

International Standard of Œstrogen Activity.—This is expressed in terms of a *mouse unit* (m.u.), which is defined as the œstrogenic effect of 0.1γ of a standard preparation of *œstrone*.

Œstradiol is the actual ovarian follicular hormone. It is a colourless steroid, slightly soluble in water, but freely soluble in organic solvents. Chemically, it is the 3:4-dihydroxy derivative of *œstrane*, or 13-methyl *cyclo-pentano phenanthrene*, the mono-methylated steroid nucleus which occurs in all the natural œstrogens (p. 172). *Œstrone* and *œstriol* represent various oxidation

The Natural Œstrogens

Name.	Formula.	Sources.	Discoverer.	Relative Potency of 0.001 m.g. (1γ).
(1) Œstradiol	$C_{18}H_{24}O_2$	Ovary.	Doisy, 1935.	20 m.u.
(2) Œstrone (Theelin) (Œstrin)	$C_{18}H_{22}O_2$	Ovary. Urine. Palm kernel.	Doisy, 1929. Butenandt, 1929. Dingemans, 1929.	10 m.u. (= standard)
(3) Œstriol (Theelol)	$C_{18}H_{24}O_3$	Placenta. Urine. Willow tree.	Collip, 1930. Marrian, 1930.	0.1 m.u.
(4) Equilin	$C_{18}H_{20}O_2$	Urine.	Girard, 1930.	1.0 (?)
(5) Equilenin	$C_{18}H_{18}O_2$	Urine.	Girard, 1930.	0.5 (?)

forms in which œstradiol is excreted, after esterification, in the urine. The most active of these compounds is œstradiol, and the least active is œstriol, which on account of its three hydroxyl groups is more soluble in water and less soluble in fat-solvents than the other œstrogens. Œstriol is manufactured mostly in the placenta, during pregnancy, where it occurs partly as a glucuronide, *emmenine*.

Physiological Effects of Œstrogens.—In experimental animals, whose ovaries have been removed, injection of an œstrogen causes rapid growth of the vaginal epithelium, as in normal œstrous states, together with increased growth of the uterine mucosa and the mammary tissue. In the normal animal, œstrogen injection hastens the onset of œstrus. These effects are accompanied by (i.) increased metabolism, with loss in weight, especially in obese gonadectomised animals; (ii.) glucose mobilisation and increased resistance to insulin.

Excessive doses of natural or artificial œstrogens inhibit the gonadotropic activity of the pituitary gland (Noble, 1938).

The Luteal Hormone

The corpus luteum, which is formed from the ovarian follicle after escape of the ovum, elaborates a hormone, *progesterone*, or *progestin*, that in the human non-pregnant condition acts by stimulating the growth and secretion of the endometrial mucosa during the fourteen days prior to menstruation. In the pregnant condition, the corpus luteum persists, maintained by an autacoid secreted by the embryo, and the output of progesterone continues until parturition. Thus, ovarian activity is associated with the production of two independent hormones, œstradiol and progesterone.

Progesterone, or *luteosterone*, $C_{21}H_{30}O_2$, the luteal hormone, was

recognised by Corner, Allen, Gley and other independent workers, and obtained in pure crystalline form by Butenandt. It is a methoxy-keto derivative of a dimethyl steroid nucleus similar to that found in the androgens, and is responsible for the three principal functions of the corpus luteum :—

(1) Premenstrual endometrial growth and secretion in the non-pregnant state.

(2) Inhibition of ovulation and menstruation during pregnancy.

(3) Embedding of the fertilised ovum, and placenta formation.

Significance of the Ovarian Hormones.—Extirpation of the ovary before puberty inhibits the development of the secondary sexual characters and the establishment of the menstrual cycle. Extirpation after puberty induces the syndrome characteristic of the menopause, or climacteric, which in the human subject normally occurs between the ages of forty-five and fifty, and is often associated with a general endocrine disturbance. Ovarian grafts in the ovariectomized subject lead to a temporary restoration of the sexual cycle, but the implanted tissue degenerates usually within a year. Ovarian grafts in the normal male animal are unsuccessful owing to the antagonising effect of a testicular factor. If, however, the animal is previously castrated, the ovarian graft displays a feminizing effect, as shown by growth of the mammary glands and partial assumption of the secondary female sex characters. Oestrogens repeatedly injected into normal male animals evoke prostatic hypertrophy and enlargement of the utriculus, which is the homologue of part of the genital tract in the female.

On account of the importance of the ovary, it was formerly assumed that the tissue was more or less autonomous, and produced the hormones automatically; but it is now known that ovarian activity is completely subservient to the control of the anterior pituitary gland, and that many conditions ascribed to ovarian incompetence are due to pituitary dysfunction (Parkes, 1930).

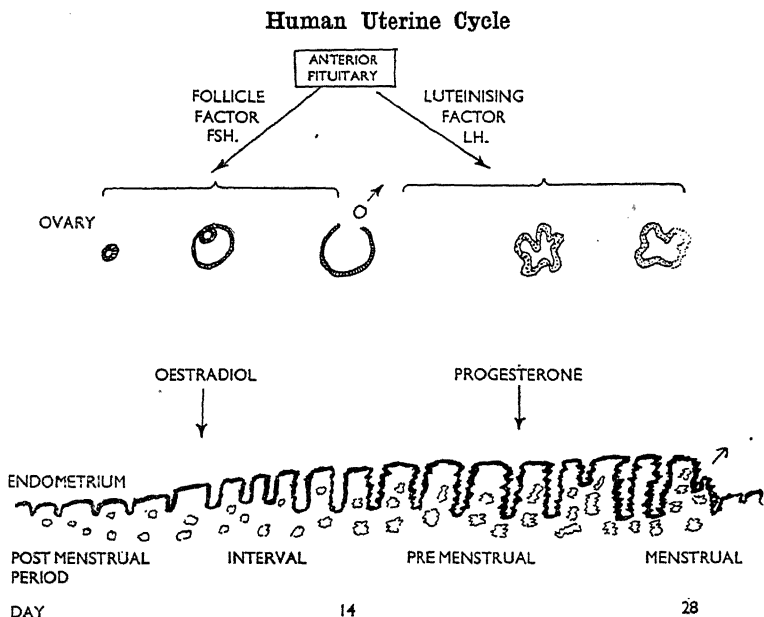
Pituitary Factors in Ovarian Activity.—The anterior lobe of the pituitary gland secretes two factors, which on account of their action on the sex glands are called *gonadotropic* hormones.

(1) *Follicle-stimulating Hormone* (FSH), “prolan A,” or factor A, promotes development of the Graffian follicle in the ovary.

(2) *Luteinising Hormone* (LH), “prolan B,” or factor B, brings about formation of the corpus luteum. FSH and LH are hormone proteins, FSH occurs chiefly in acid extracts of the anterior pituitary, and LH in alkaline extracts.

Rupture of the follicle and liberation of the ovum is also controlled by the anterior pituitary, perhaps by the sudden onset of secretion of LH.

Thus, it appears that the uterine cycle in non-pregnant and in pregnant conditions is controlled by the operation of two distinct groups of hormones, as shown in the following diagram (Jeffcoate, 1935).



The Menstrual Cycle

Days.

- Secretion of FSH induces secretion of oestradiol by granulosa cells of the follicle.
- 5-11 Rapid growth of follicle. Continued secretion of FSH and oestradiol. Growth of the endometrium.
- 12-14 Rupture of follicle and escape of ovum. Secretion of LH. Formation of the corpus luteum.
- 15-26 Continued secretion of LH. Persistence of corpus luteum. Secretion of progesterone. Enlargement and secretory activity of endometrial glands.
- 27 Inhibition of pituitary hormones by the increase in the concentration of oestradiol. Corpus luteum starts to degenerate.
- 28 Onset of menstruation. Cessation of secretion of FSH. Rapid degeneration of corpus luteum. Decline in secretion of progesterone and oestradiol.

- 1-4 Decrease in secretion of ovarian hormones leads to renewal of pituitary activity, and continuation of the cycle.

Hormones in the Urine of Pregnancy.—During pregnancy, the persistence of the corpus luteum is assured by the continued secretion of LH, with the result that three types of hormones appear in the urine: (i.) Œstrogens, which are excreted in the inactive esterified form, so as to protect the foetus from the powerful hormones of the mother; (ii.) progesterone, excreted in the inactive form of pregnandiol; (iii.) gonadotropic pituitary hormones.

In human pregnancy urine, the hormone is chiefly œstrone; in urine of the cow or mare, it is chiefly œstriol. Both œstrogens are excreted as inactive esters of glycuronic acid, until the onset of labour, when they appear in the free form (Cohen and Marrian, 1935).

Small quantities of œstrogen appear in human urine about the fifteenth day of the menstrual cycle, and disappear just before the onset of menstruation. During pregnancy, the output rapidly increases to a maximum of 1.5–2 mg. per litre, which persists till parturition, and then rapidly falls to the normal minimum.

Œstrogens and Pituitary Gonadotropic Hormones in Urine
(Representative values, expressed in mouse units)

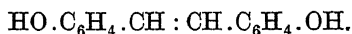
	Œstrogens.				Gonadotropes, per Litre.	
	Non-pregnant.		Pregnant.			
	Per Litre.	Per Diem.	Per Litre.	Per Diem.	FSH.	LH.
Woman .	425	600	21,000	31,000	20,000	10,000
Man .	160	240	—	—	—	—
Mare .	200	2,000	100,000	1,000,000	300	0
Stallion .	170,000	1,700,000	—	—	—	—
Bull .	330	—	—	—	—	—

The appearance of œstrone in pregnancy urine is due to the non-utilisation of the hormone during the suspension of the œstrus cycle. In the non-pregnant state, the hormone is required for the maintenance of the cycle, and only its derivatives, the diols, appear in the urine. During pregnancy, no appreciable amount of progesterone is excreted, as it is being used in the control of uterine development.

Equilene and equilenine occur as reduction derivatives, along with œstrone in the pregnancy urine from the mare, which has a total

oestrogen value of about 10 mg. per litre, or about ten times as great as that of human pregnancy urine. Stallions differ from other male animals in excreting a urine very rich in oestrogens, the hormones having been formed in the testicle along with the androgens.

Synthetic Oestrogens.—Dodds and other workers have prepared a number of cyclic compounds capable of evoking oestrus, the most potent being derivatives of 4:4'-dihydroxystilbene, a parent hydrocarbon which Dodds terms "stilbcestron" (1938),



This discovery shows that the steroid ring is not necessary for oestrogenic function. The derivative *diethyl stilbcestron* is about two and a half times more potent than oestrone, but not as active as oestradiol.

Standardisation of the Oestrogens.—The original standard was the rat unit (r.u.) or the mouse unit (m.u.), which was defined as the minimal quantity of hormone necessary to evoke oestrus in the gonadectomised animal, the state being recognised histologically by the Allen and Doisy test. These units are apt to vary according to differences in laboratory technique, and a committee established by the League of Nations has recommended an international unit equivalent to 0.1 γ (0.0001 mg.) of crystallised oestrone ("oestrin").

Sources of the Oestrogens.—Oestradiol and oestrone can be obtained from ovarian tissue of domestic animals, and display no species specificity. That these or other oestrogens can arise independently of the follicle is shown by the fact that the oestrus cycle can persist after complete destruction of the ovarian follicles by short-wave irradiation, and also by the persistence of oestrogens in pregnancy urine after ovariectomy. The placenta and the adrenal cortex have been suggested as extra-ovarian sources of the hormones. Commercially, oestrogens are now obtained in quantity from the urine of the cow or the horse, an unexpected discovery being that the female sex hormones are excreted both in male and in female urine, and, conversely, the male sex hormones have been isolated from female urine. This paradox is explained by the close chemical relationship between both groups of compounds, each member of which is derived from a methylated steroid nucleus. Vegetable sources are represented by palm kernels and palm oil.

Biological Tests for Pregnancy.—The detection of the placental gonadotropic factors in urine constitutes a very delicate and trustworthy test for pregnancy. The test consists of the injection of the urine into young mice (Aschheim-Zondek test), rabbits

(Friedman test), or a species of toad, *Xenopus laevis* (Hogben test), and the observation of subsequent histological changes.

Aschheim-Zondek Test.—Five mice, aged three to four weeks, and weighing 6–8 gm. each, are injected individually with 1.2–2.4 ml. of urine, divided into six doses given during three days. All are killed on the fifth day. A positive result for the urine is claimed if at least one animal shows ovarian changes, notably the presence of corpora lutea and corpora hæmorrhagica. Positive results have been obtained with urine as early as the fifth week of pregnancy.

The test will distinguish between true pregnancy and menopausal conditions, including “endocrine repercussions of abnormal emotional states.” It is advisable to extract the urine with three times its volume of ether prior to injection in order to remove a toxic substance present in some urines, and fatal to mice.

The Friedman test only requires twenty-four to forty-eight hours, and for this reason is sometimes preferable. The relative merits of the three tests are discussed by Crew (1939).

ANDROGENS: MALE SEX HORMONES

The existence of chemical factors controlling secondary sexual characters in the male had long been inferred from the more obvious consequences of castration, before or after puberty. Early attempts by Brown-Séquard and others to obtain active extracts from testicular tissue were inconclusive, mainly for two reasons: the preparations were administered by the alimentary tract, from which the hormones are only absorbed with difficulty; and no exact method of chemical assay was available. Since 1930, Moore and others have shown that androgenic activity may be detected by the increased growth of the comb and wattles in castrated birds (capons); and Korenchevsky has based a method of assay on the increased growth of the prostate and seminal vesicles evoked when the hormone is injected into castrated rodents. Aided by these tests, four natural androgens have been isolated, and various artificial androgens have been synthesised.

Natural Androgens

Name.	Formula.	Source.	Discoverer.	Potency, in Rat Units.
Androsterone . .	$C_{19}H_{26}O_2$	Male urine.	Butenandt.	1 mg. = 1 r.u.
Dehydroandrosterone	$C_{19}H_{24}O_2$	Male urine.	Butenandt.	1 mg. = 0.3 r.u.
Testosterone . .	$C_{19}H_{28}O_2$	Testicle.	Laqueur.	1 mg. = 0.1 r.u.
Adrenosterone . .	$C_{19}H_{26}O_3$	Adrenal cortex.	Reichstein.	—

Testosterone, $C_{19}H_{28}O_2$, the testicular hormone, is an unsaturated hydroxy-ketone derived from a parent steroid *androstan*e, and appears in the urine as the two derivatives, *androsterone* and *dehydroandrosterone*, both of which are much less potent.

A fourth androgen, *androstandiol*, $C_{19}H_{28}O_2$, can be prepared by reduction of androsterone, and has about one-third the potency of the parent compound.

Testosterone and androsterone can now be manufactured in quantity from cholesterol and other common sterols, which provide a more convenient alternative to the natural sources.

The androgens are all colourless crystalline compounds, sparingly soluble in water but readily soluble in fat solvents, and are usually administered by intramuscular injection of the solution in an oil.

The international unit recommended by the League of Nations (1935) is the hormone activity of 0.1 mg. of crystalline androsterone, as tested by a specific biological reaction. A 15 per cent. increase in the comb area may be expected from administration to a capon of one international unit daily for five days.

Effects of Androgen Injection.—In the castrated mammal there is a specific growth of accessory genital glands, the prostate, seminal vesicles and glands of Cowper, and a manifestation of the secondary sexual characters. The androgens differ somewhat in their results; androstandiol is about twice as active on the prostate as androsterone, and about four times as active on the seminal vesicles. Testosterone, the most generally active, produces in capons twice as much comb growth as androstandiol and about six times as much as androsterone.

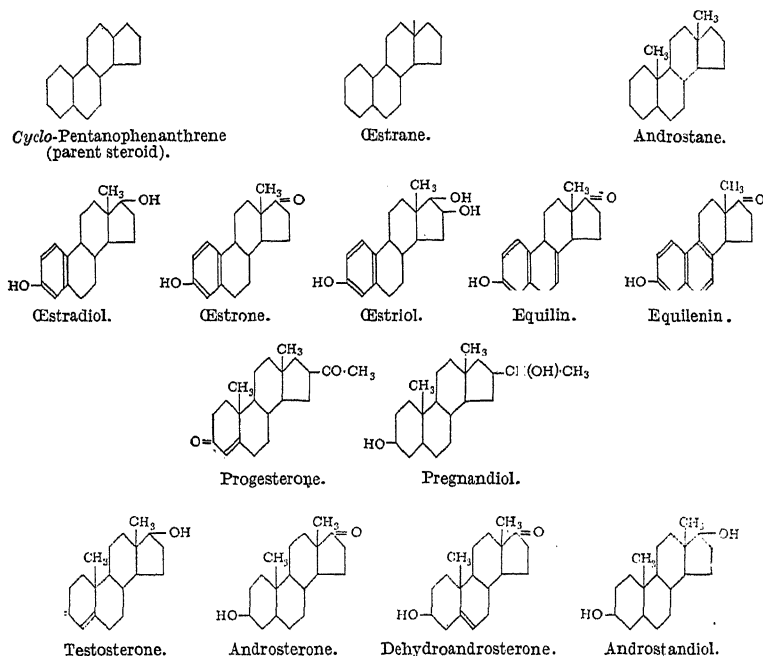
Significance of the Androgens.—Castration before puberty induces the eunuchoid state in which the secondary sexual characters fail to develop. Castration after puberty has little obvious effect other than subjective changes following the sterilisation. From this it might appear that the function of the androgens is limited to the maintenance of the reproductive efficiency of the individual. The isolation of an androgen, *adrenosterone*, among the hormones of the adrenal gland, suggests that extra-testicular sources may be available in the adult.

The Pituitary Control of Testicular Activity.—Gonadotropic hormones secreted by the anterior pituitary control (i.) the descent of the testicle, (ii.) the onset and maintenance of spermatogenesis, and (iii.) the output of the androgens, this last effect being homologous with the action of FSH in the female. Pituitary gonadotropic activity is in abeyance before puberty, as shown by the absence of androsterone from the urine.

The oestrogenic female hormones (gynæcogens) are all derived

from the 13-methyl steroid *œstrane*, $C_{17}H_{27}\cdot CH_3$. Progesterone, pregnandiol and the male hormones are all derived from the 10:13-dimethyl steroid androstane, $C_{17}H_{26}(CH_3)_2$.

STRUCTURE OF THE SEX HORMONES



PITUITARY AUTACOIDS

The pituitary body, or hypophysis, is double in origin and multiple in functions. From the stomodœal pouch of Rathke is developed the anterior lobe and the *pars intermedia*; from the floor of the third ventricle are formed the *pars nervosa* of the posterior lobe, the *infundibulum* and the *tuber cinereum*. The pituitary autacoids are of two types: secretions acting directly on other tissues, and *hormo-kinetic* secretions acting indirectly by stimulating other endocrine glands.

A. Anterior Lobe

(1) **Pituitary Growth Autacoid, Phyone** (Van Dyke and Wallen-Lawrence, 1930), "Tethelin" (Robertson, 1915).—This factor

stimulates general growth, particularly that of the osseous, connective, and epithelial tissues. It has no obvious action on the thyroid, the ovaries, or any other endocrine structure. It is lipoid in character, and is usually present in alkaline, but not in acid, extracts of the anterior lobe. Lack of the factor causes infantilism; excess causes gigantism and acromegaly.

Female Gonadotropic Hormones, FSH and LH

(2) **Oestrogenic Factor**, FSH, Prolan A (Zondek, 1930).—This evokes the secretion of oestradiol, and thus determines the onset of puberty, the maintenance of the menstrual cycle and the sequence of events in pregnancy. It is present in acid extracts of the anterior gland, and is excreted in the urine of pregnancy, along with the oestrogen from the placenta (chorionic gonadotropin) that is responsible for the Aschheim-Zondek reaction.

(3) **Luteinising Factor**, LH, Prolan B (Zondek, 1930).—This evokes growth of the corpus luteum. It is present in both acid and alkaline extracts of the gland, and is excreted in the urine of pregnancy.

(4) **Prolactin**.—The growth of the mammary glands during pregnancy is ascribed to the action of the oestrogens; the actual onset of lactation is due to a pituitary factor, the effects of which can be demonstrated in the ovariectomised animal, showing that they are independent of oestrogen once the mammary gland has developed. Prolactin accompanies LH in alkaline extracts of the anterior pituitary.

The male gonadotropic factors are the counterparts of the follicle-stimulating and luteinising hormones in the female, and await fuller investigation.

(5) **Thyrotropic Principle** (Crew and Wiesner, 1930) stimulates thyroid development in young animals, and regulates the secretion of the gland. Hyperthyroidism or hypothyroidism may result from excessive or inadequate output of the thyrotropic principle.

(6) **Adrenotropic Principle**. Hypophysectomy leads to atrophy of the adrenal cortex as well as atrophy of the thyroid. Pituitary hyperfunction, as seen in gigantism and basophil neoplastic growths in the gland, is often associated with cortical hypertrophy and with hyperthyroidism. Whether these effects are due to independent thyrotropic and adrenotropic hormones, or to a single hormokinetic hormotropic principle is not yet certain.

(7) **Metabolic Principle** (O'Donovan and Collip, 1938).—Even in thyroidectomised animals injection of anterior pituitary extracts may stimulate metabolism, as shown by increased oxygen con-

sumption and rise in body temperature. This is accompanied by a fall in the respiratory quotient, showing that there is a suppression of carbohydrate oxidation.

(8) **Pancreotropic Principle** regulates insulin secretion or action in some way not yet understood.

(9) *Ketogenic and Glycotropic (diabetogenic) principles* are claimed to exist, and have been discussed along with carbohydrate metabolism (p. 300). The ketogenic principle is believed to stimulate the production of β -hydroxy butyric acid and the ketones found in diabetic urine. The glycotropic principle antagonises the effect of insulin, and consequently raises the level of the blood sugar. How far these effects are independent of the action of the metabolic principle is not known, and all of them may be due to the action of a single hormone working under different metabolic conditions.

Significance of the Anterior Pituitary Gland.—By means of the gonadotropic hormones, FSH and LH, the gland regulates the œstrus cycle. By means of the hormotropic principles, it co-ordinates the activity of the thyroid, adrenal and pancreas. By means of the growth factor and metabolic principle, it regulates development and general metabolism.

B. Posterior Lobe (*pars intermedia* and *pars nervosa*)

(1) **Anti-diuretic Factor**, anti-diuretin.—Selective removal of the posterior pituitary evokes a polyuria due to inability of the renal tubule to concentrate the glomerular filtrate. A very dilute urine is excreted, unaccompanied by any abnormal solutes, although the total sodium chloride output is increased in the early stages of the diuresis. Pathological pituitary dysfunction is associated with the disease diabetes insipidus, in which the urinary output may exceed 8–10 litres *per diem*. This leads to an acute dehydration of the organism and an attendant abnormal thirst, or *polydypsia*. Both experimental and pathological forms of the diuresis are rapidly abolished by injection of posterior pituitary extract. The recovery is transient, owing to destruction or excretion of the anti-diuretic factor. The factor has been separated from normal urine by Gilman and Goodman (1937), and shown to be distinct from the other pituitary principles. It is a true hormone, and is secreted by the gland in accordance with the need for water conservation.

(2) **Oxytocin**, α -hypophamine (Kamm *et al.*, 1928), causes a powerful contraction of the uterine muscle, and also stimulates the musculature of the bladder, the intestine, and the rest of the unstriated muscle of the organism, with the exception of that of the

bronchi. A pseudo-galactagogue effect is also seen in the temporary outpouring of milk that follows injection of oxytocin or of pituitary extracts. This is ascribed to the expression of preformed milk from the gland owing to contraction of the ducts.

(3) **Vaso-pressin**, β -hypophamine, causes contraction of capillaries and arterioles after injection. The blood pressure slowly rises and remains high for several hours. There is little change in the rate and force of the heart beat other than that due to increased vascular resistance.

Injection of a second dose after the pressor effects of the first have worn off usually causes little or no response, the vascular system remaining insensitive for several hours. This may be due to the appearance of an anti-vaso-pressin in the organism, or, more likely, to the exhaustion of a contractile factor in the vessel walls. Birds for some unexplained reason appear to be immune from the vaso-pressor action of pituitary extracts. In man, the constricting effect on the capillaries is very conspicuous in the blanching of the skin that follows subcutaneous injection.

Apart from their therapeutic value, neither oxytocin nor vaso-pressin can be regarded as true hormones, since there is no evidence that they are employed in the regulation of physiological activity. Pregnancy and parturition are unaffected by removal of the posterior pituitary, and the normal uterine contractions during labour differ in character from those evoked by oxytocin. No marked circulatory disturbances follow removal of the posterior pituitary, and, although extracts containing vaso-pressin are active when administered to anæsthetised animals, little or no pressor effect is observed in unanæsthetised dogs and human subjects. Commercial preparations of the posterior pituitary rich in one or more of the characteristic principles are represented by "pituitrin," "pitocin" (oxytocic), "pitressin" (vaso-pressor), and "infundin."

(4) **Chromotropic Factor**.—The pigmentary system of amphibians is controlled by a pituitary autacoid. Injection of posterior gland extracts into adult frogs evokes a rapid darkening of the skin owing to dilation of the pigment cells, or melanopores.

Hypopituitarism.—This may involve anterior or posterior lobe deficiency, or the entire gland.

(1) *Anterior Hypopituitarism*.—*Acute*.—Evidence is still inconclusive as to whether the anterior lobe is immediately essential for life.

Chronic, as seen in maldevelopment or atrophy, is characterised by (i.) dwarfism, (ii.) sexual infantilism, (iii.) obesity, with lowered basal metabolic rate. The condition is profoundly modified by the fate of the thyroid gland.

(a) Fröhlich's syndrome, dystrophia adiposo-genitalis, occurs in children, and is due to infantile hypopituitarism and hypothyroidism. The victims are stunted, stupid, and often distorted by diffuse deposition of fat.

(b) Lorain's syndrome. Hypopituitarism unaccompanied by hypothyroidism. There is retardation of skeletal growth and absence of secondary sexual characters. Mental ability is unimpaired, and is often much above the normal.

Hypopituitarism is frequent in old age, and marks a stage in senility.

(2) *Posterior Hypopituitarism.—Acute.*—There is no convincing evidence that the posterior lobe is essential for life. Complete removal is not followed by specific symptoms of deficiency. This paradoxical result may be due to (i.) readjustment of the organism during convalescence to a lower autacoid requirement, or (ii.) compensatory secretion by other endocrine organs, or (iii.) undetected persistence of sufficient *pars intermedia* tissue to provide for the requirements of life.

Chronic.—The only clinical condition ascribed to chronic dysfunction is diabetes insipidus. Krogh has suggested that vasopressin is necessary for the maintenance of capillary tone, and that hypopituitarism is associated with low blood pressure, but Dale concludes that the adrenal medulla is the principal if not the exclusive factor in determining the condition.

Hyperpituitarism.—This may be of the anterior or posterior type, according to the tissue involved.

(1) *Anterior hyperpituitarism* :—

(a) Gigantism, due to early hyperpituitarism before the epiphyses of the long bones have united. The bones continue to grow uniformly, and the skeleton may reach a height of 7 or 8 ft.

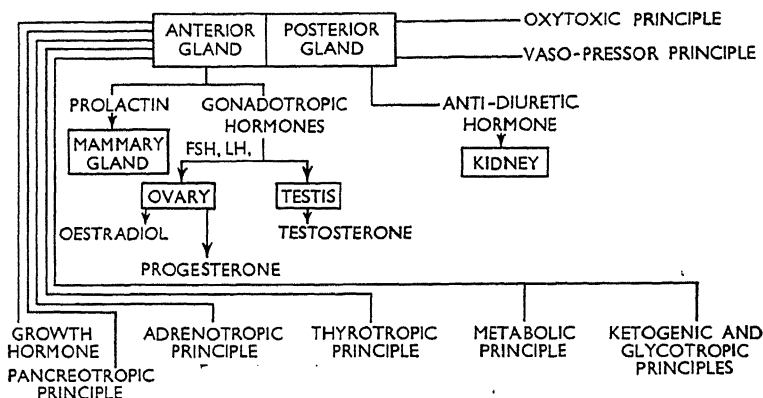
(b) Acromegaly, due to late hyperpituitarism after the epiphyses have united. There is little change in the height of the skeleton, instead there is local overgrowth of the bones of the lower half of the face, enlargement of the hands and feet, curvature of the spine, and an approximation to the "gorilla appearance." There is hyperplasia of the connective tissue and thickening of the skin. The sexual potential is increased, but usually decays prematurely.

Both gigantism and acromegaly may be complicated by hypersecretion of the posterior lobe, leading to erratic glycosuria.

(c) Pregnancy is marked by increasing secretion of the anterior sex autacoids, and their appearance in the urine.

(2) *Posterior hyperpituitarism* has not yet been recognised as a clinical entity, although it may be involved in miscarriages and premature labour, and, perhaps, in some forms of anuria.

Pituitary Autacoids



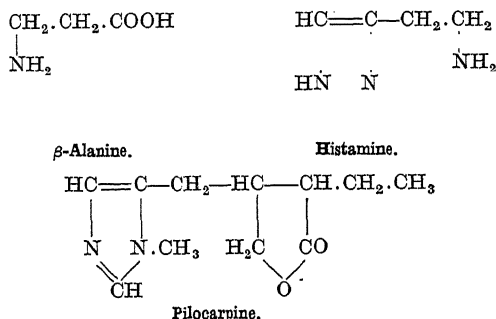
ALIMENTARY HORMONES

The events in the digestive process are controlled partly by the nervous system, and partly by hormones liberated by the action of the digestive products on the gastric and intestinal mucosa.

(1) **Gastrin**, gastric secretin.—In 1905, Edkins showed that acid extracts of the pyloric mucosa, when neutralised and injected into animals, evoked a secretion of gastric juice, an effect he attributed to the presence of a specific secretagogue, *gastrin*. Subsequently it was found that meat juice, meat extracts, protein digestion products and certain amino acids had a similar action, indicating that these factors might be of considerable importance in the maintenance of gastric digestion. The most potent secretagogue effect, however, was displayed by histamine and by β -alanine, neither of which are constituents of the native proteins. β -Alanine is a component of carnosine and of anserine which occur in muscle tissue (p. 374), and appears to be responsible for the secretagogue property of meat extracts and broths. Histamine is readily formed from the natural amino acid histidine, by decarboxylation, and, according to Ivy (1933), is the actual gastric hormone present in pyloric mucosa, and is liberated during digestion.

Histamine is now employed for the purpose of obtaining human gastric juice for fractional analysis. About 0.1 mg. per 10 kg. of body weight is injected; gastric secretion begins within five minutes, and reaches a maximum between thirty to forty-five minutes after the injection. The juice evoked by histamine is rich in hydrochloric acid but deficient in enzymes, which indicates that the amine acts preferentially on the parietal cells. Injection of pilo-

carpine evokes a secretion rich in enzymes and mucin, and when administered along with histamine results in the production of a gastric juice similar to that secreted during normal digestion.



(2) **Secretin**, the secretory hormone for the pancreas, is found in the mucosa of the upper two-thirds of the small intestine. It can be extracted by acids, soaps, 70 per cent. alcohol, and 0.6 per cent. NaCl. All these methods have the disadvantage that they yield a product highly contaminated with vaso-dilators, during the subsequent removal of which most of the secretin is lost. The purified autacoid is a white powder containing 6.7-8 per cent. N, and resembling a peptide in its reactions. When injected intravenously it causes a rapid secretion of pancreatic juice, and also has a secretagogue effect on the liver and the glands of the small intestine. The action is not species specific, and somewhat resembles that of pilocarpine, but the pancreatic secretion evoked by secretin is richer in alkali and poorer in enzymes than the secretion obtained by vagal stimulation or the use of a vagomimetic drug, such as pilocarpine.

Secretin Distribution in Alimentary Mucosa of the Cat, expressed in Volume of Pancreatic Secretion evoked by a Standard Dose

Region.	Secretion.
	Ml.
Stomach, fundus . .	0.0
„ pylorus . .	0.0
Small intestine :	
Upper third . .	2.75
Middle third . .	2.6
Lower third . .	0.5
Ascending colon . .	0.2

The autacoid is inactive when given by the mouth, which greatly restricts its therapeutic application.

Secretin is liberated by the presence of bile salts, fats, or to a much lesser extent, free acid, in the upper small intestine.

The secretin unit suggested by Ivy is "that amount of dried material in solution which when injected will cause a 10-drop (0.4 ml.) increase in the rate of flow of pancreatic juice within a ten-minute period following the time of injection, as compared with the previous preceding ten-minute period."

The animal used for the purpose of assay is the cat.

(3) **Cholecystokinin**, a hormone evoking contraction of the gall bladder, resembles secretin in its distribution, and is present in extracts of the intestinal mucosa. Separation of the two hormones has been claimed by Ivy and Oldberg (1928).

MISCELLANEOUS AUTACOIDS

Extracts of animal or plant tissues when injected into higher animals often display a vaso-pressor effect owing to the presence of *vaso-dilatins* acting on arterioles and capillaries. Some of these vaso-dilatins appear to be artefacts formed during the preparation of the tissue extract, but at least six are believed to exist as biological reactants, and may be regarded as typical hormones. These are: (1) Histamine, (2) acetyl choline, (3) choline, (4) adenylic acid, (5) kallikrein, and (6) testicular diffusing factor.

(1) **Histamine** has been identified in extracts of intestinal mucosa, liver, lung and posterior pituitary. It displays three characteristic effects:—

- (i.) A secretagogue action on the lachrymal, salivary, gastric and pancreatic glands.
- (ii.) Contraction of involuntary muscle, notably uterus, intestine and bronchioles.
- (iii.) Fall in blood pressure owing to generalised capillary dilatation and paralysis, accompanied by increased capillary permeability and transudation of plasma. In the human subject, a subcutaneous injection of 0.3 mg. of histamine evokes a marked fall in diastolic pressure, a rise in pulse rate and temperature, and a flushing of the skin. Arteriolar dilatation accompanies the capillary response in man, dogs and monkeys.

(2) **Acetyl choline**, the most powerful reactant known to occur in the animal body, has been identified in fresh spleen by Dale and Dudley, and also occurs as the neurocrine liberated locally when

parasympathetic (cholinergic) nerve endings transmit impulses. Characteristic effects following intravenous injection are :—

- (i.) Fall in blood pressure owing to a direct dilator action on peripheral blood vessels. This effect is observed even in doses of 1×10^{-5} mg., and is antagonised by atropine.

The direct vascular effect is accompanied by cardiac inhibition equivalent to vagal stimulation.

- (ii.) *General Cholinergic Phenomena.*—These are similar to the effects of parasympathetic stimulation, and include lachrymal, salivary, gastric and pancreatic secretion, increased motility of the alimentary tract, and contraction of the bladder.

Acetyl choline is transient in effect owing to its rapid hydrolysis by the widely distributed enzyme, choline esterase, which resolves it into free choline, with a potency about 100,000 times less than the acetyl ester. Choline esterase is inhibited by eserine (physostigmine), application of which prolongs the action of acetyl choline.

(3) **Adenylic acid**, present in extracts of skeletal and cardiac muscle, and in brain, kidney and spleen, is derived from adenosine triphosphate, the phosphate carrier in glycogenolysis and glycolysis. Adenylic acid, and its nucleoside component adenosine, are active vaso-depressors, evoking general arterial dilatation accompanied by cardiac retardation. The coronary arteries are dilated, and for this reason preparations of the autacoid ("lacarnol") have been used in the treatment of angina pectoria. Accumulation of adenylic acid in the tissues is prevented by its conversion into the less potent nucleotide inosinic acid (p. 348).

(4) **Kallikrein**, or angioxyl, a colloid capable of reducing blood pressure and antagonising the pressor effect of adrenaline, has been separated from extracts of the pancreas (Frey and Kraut, 1928; Gley, 1929), and has been used therapeutically ("padutin") for the treatment of vascular disorders. Kallikrein accompanies the FSH fraction in urine, and resembles these pituitary hormones in physical properties. Its physiological significance is doubtful (Bischoff and Elliot, 1937).

Testicular Diffusing Factor.—Aqueous extracts of mammalian testicle contain a substance that greatly increases skin permeability to injected fluids, and the injection spreads rapidly through the dermis. Intracutaneous injection of saline, serum or extracts of any tissue other than testicle cause a bleb, the margins of which persist for twenty to thirty minutes.

PLANT AUTACOIDS, PHYTOHORMONES

Name.	Source.	Function.
(1) Bios complex . . .	Yeast extracts, bran, etc.	Growth factor.
(2) Auxin <i>a</i> . . .	Apical tissue of shoots, etc. Pollen.	Cell elongation. Phototropism. Geotropism.
(3) Auxin <i>b</i> . . .	Plant extracts.	Growth factor.
(4) Indole-3-acetic acid	Urine, etc.	Root formation.
(5) Traumatic acid . .	Injured tissue.	Wound repair.

(1) **Bios Complex.**—In 1901, Ide and his pupil Wildiers showed that a water-soluble factor, termed *bios*, is necessary for the growth and development of many, but not all, strains of the yeast *Saccharomyces cerevisiae*. Bios occurs plentifully in yeast extracts, plant leaves, bran and the outer coats of seeds. In many ways it resembles a vitamin complex, but since it is synthesised by the growing yeast it is now included among the autacoids. Various compounds have been isolated from the mixture of substances present in bios extracts, and their specific potency has been accepted or denied by different workers.

These conflicts of opinion are now regarded as due largely to the differences in species effect, which have led to ambiguous or contradictory results. The growth of all monocellular organisms depends on the elaboration of cyto-skeletal compounds, and growth rate can be increased when some at least of these are provided by the environment.

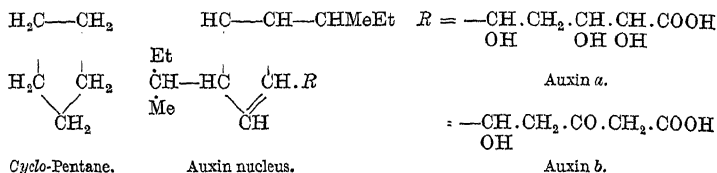
A bios may be defined as a growth factor for monocellular organisms that can be derived both from endogenous and exogenous sources. Representative bios substances are: (i.) *i*-inositol, or bios I.; (ii.) nicotinic acid, which is necessary for the growth of *Staphylococcus* and other bacteria; (iii.) uracil; (iv.) vitamin B₁; (v.) panthothenic acid, a polyhydroxylic acid derived from β -alanine, and necessary for yeast growth, and (vi.) biotin, a sulphur-containing factor isolated by Kögl.

(2) **Auxins.**—Plant development is attended by elongation of individual cells, as distinct from their multiplication, and by resulting movements of stems towards the light (phototropisms) or roots towards the soil (geotropisms). All these changes have been shown to be due to hormones, termed auximones or auxins, secreted in the apical regions and spreading by diffusion.

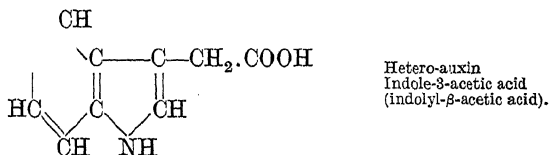
The study of auxins arose, in 1910, with the work of Boysen-Jensen on the coleoptile, or primary leaf sheath, of grasses, and was extended by Went, and other investigators; the isolation and identification of the auxins being due to Kögl and his colleagues (1931-37).

Auxin *a* and auxin *b*, the principal phytohormones, are obtained from the ether-soluble fraction of lipid extracts of rapidly growing apical tissues of roots and tips, and are assayed in terms of their ability to evoke renewed growth in decapitated coleoptiles (auxin *a*) or in moulds (auxin *b*). Auxin *b* is readily destroyed by both acids and alkalis, and thus can be separated from auxin *a*, which is relatively acid-stable, but decomposed by alkalis.

Both the auxins are derivatives of a *cyclo*-pentane ring similar to that forming part of the steroid nucleus.



(3) **Hetero-auxin, Indole-3-acetic Acid.**—In the survey of plant and animal extracts for substances having an auxin effect, it was found that urine was very rich in a growth-promoting factor, which on isolation proved to be chemically unrelated to auxin *a* or *b*, and was termed hetero-auxin. It was subsequently identified as an indole derivative of acetic acid, and is derived from tryptophane by bacterial decomposition in the intestine.



The effect of the phytohormones is non-specific as regards species ; auxin *a* stimulates growth in a great variety of plants ; auxin *b* has no action on coleoptiles, but promotes growth and mycelium formation in *Aspergillus niger*, and other moulds ; hetero-auxin appears to be a general stimulant for root formation. None of these hormones has been found to influence cultures of animal or malignant tissues, although there is a quantity of miscellaneous evidence that animal hormones, notably the cestranes and thyroxin, can promote plant growth.

(4) **Traumatic Acid**, the plant wound hormone, is liberated by damaged tissues, and stimulates growth of parenchymatous cells in the repair process. It has been isolated by English (1939) and shown to be 1 — decene — 1, 10 — dicarboxylic acid, $\text{HOOC} \cdot \text{CH} = \text{CH} \cdot (\text{CH}_2)_7 \cdot \text{CH}_2 \cdot \text{COOH}$.

ORGANISERS AND EVOCATORS

Embryonic development involves two processes: *differentiation* and *organisation*. As growth proceeds, various territories of the segmented egg lose their common primordial character, and acquire the morphological and chemical features of tissues and organs. "Viewed from the community of parts, this is differentiation; viewed from the individual parts, it is specialisation" (Weiss, 1935). These processes of differentiation or specialisation are accompanied by organisation, whereby the emergent structures are arranged in accordance with the species pattern of the individual. Formerly, embryonic development was popularly and vaguely attributed to a teleological guidance or purpose latent in the embryo, but since 1921 the work of Spemann, Mangold, Waddington and the Needhams has shown that it depends on the activity of chemical factors, or *organisers*, elaborated locally and exerting an *inductive* effect by diffusion into neighbouring sensitive areas, or *fields*. Induction may occur naturally during the growth process or be brought about artificially by means of grafts. Two types of graft induction can occur: *evocation*, in which the graft carries no field of its own, and merely activates a field in the affected region of implantation; and *induced organisation*, in which the graft carries its own field and imposes it on the host. Under appropriate conditions, induction may be brought about by tissue extracts, as well as by fragments of living tissues, the effect being ascribed to the presence of evocators and organisers, which may be defined provisionally as morphogenetic autacoids determining embryonic development.

In the earliest stages of the developing vertebrate embryo the constituent cells have little individuality, and can give rise almost to any embryonic structure. As soon as the stage of gastrulation is reached, however, the cells become individualised, and can only give rise to particular tissues. This is due to an influence exerted on the rest of the embryo by a specialised group of cells in the region of the dorsal lip of the blastopore, which produce an organising autacoid. Removal of these organiser cells leads to cessation of normal differentiation; micro-injection of the extract of the disintegrated cells induces differentiation. It is likely that different organisers are manufactured for different purposes in embryonic development. The amphibian factor, studied by Waddington and the Needhams, appears to be a steroid. It induces the formation of the neural tube and other neural tissue in the early embryo of the newt.

GENERAL REFERENCES

- ALLEN, E. (1932), "Sex and Internal Secretions." Baltimore.
- BARGER, G. (1930), "Organic Chemistry in Biology and Medicine." New York.
- BEST, C. H., and N. B. TAYLOR (1937), "Physiological Basis of Medical Practice." London.
- BOYSEN-JENSEN, P. (1936), "Growth Hormones in Plants." London.
- BRITTON, S. W. (1930), "Adrenal insufficiency." *Physiol. Rev.*, **10**, 617.
- COLLIP, J. B. (1934), "Physiology of the anterior pituitary." *J. Mount Sinai Hosp.*, **1**, 28.
- DODDS, E. C. (1934-35), "Specificity in hormone and other biological relations." *Harvey Lectures*, **30**, 119.
- DODDS, E. C. (1937), "Sex glands." *British Med. J.*, *ii.*, 163.
- DODDS, E. C., *et al.* (1939), "Synthetic oestrogen compounds." *Proc. Roy. Soc.*, **B**, **127**, 140.
- DOISY, E. A. (1933-34), "Oestrogenic substances." *Harvey Lectures*, **29**, 158; "Endocrines in theory and practice." *British Med. J. Series*, 1937. London.
- GREGORY, J. (1936), "A B C of the Endocrines." London.
- HARRINGTON, C. R. (1933), "The Thyroid Gland." London.
- HILL, D. W., and F. O. HOWITT (1936), "Insulin." London.
- HUXLEY, J. (1935), "Chemical regulators and the hormone concept." *Biol. Rev.*, **10**, 427.
- IVY, A. C. (1930), "The role of hormones in digestion." *Physiol. Rev.*, **10**, 282.
- JEFFCOATE, T. N. A. (1935), "Sterility due to ovarian dysfunction." *British Med. J.*, *i.*, 345.
- JENSEN, H. F. (1938), "Insulin." London.
- MARRIAN, G. F. (1933), "Chemistry and biological assay of oestrin." *Physiol. Rev.*, **13**, 185.
- NEEDHAM, J. (1936), "Order and Life." Cambridge.
- NEWTON, W. H. (1938), "Hormones and the placenta." *Physiol. Rev.*, **18**, 419.
- PARKES, A. S. (1930), "The Internal Secretions of the Ovary." London.
- THIMANN, K. V., and J. BONNER (1938), "Plant growth hormones." *Physiol. Rev.*, **18**, 524.
- ZONDEK, H. (1935), "Diseases of the Endocrine Glands." London

CHAPTER XXV

THE INTERNAL ENVIRONMENT : BLOOD AND TISSUE FLUIDS

THE immediate purpose of life is survival. After a period of parasitic growth, the organism reaches a stable form capable of maintaining a biological relationship with its environment. The increasing specialisation of function associated with ascent of the evolutionary scale demands an increasing complexity of organisation and co-ordination which reaches its highest level in the human species, the most elaborate apparatus yet known in cosmic history.

But however multifarious and cryptic the activities of life, one general principle governs all physiological processes ; organic existence demands uniformity in the composition of the internal environment, which in the higher animal is represented by the blood, and the cerebro-spinal and other tissue fluids. This fundamental law has been expressed by Claude Bernard, in a well-known epigram :—

La fixité du milieu intérieur est la condition de la vie libre.

As Barcroft has shown, the freedom of human life, as expressed in repose and action, requires constancy in the composition of the blood and the cerebro-spinal fluid, while the stability of the internal environment as a whole requires inter-dependent mechanisms for storage, distribution and removal of solutes. Transitory changes in the blood due to metabolic activity are compensated for by specially adapted systems for neutralisation, detoxication, and pulmonary and renal excretion, and form part of the routine physiological activities of life. Excessive changes in the blood composition are the result of pathological processes, resulting in abnormal metabolism or in defective compensation and excretory dysfunction.

Effects of Changes in the Human Internal Environment

Deficiency. ←	Internal Environment.	→ Excess.
Hypopyrexia.	Temperature.	Hyperpyrexia.
Collapse.		Delirium.
Anoxæmia.	Oxygen.	
Unconsciousness.	H-ion concentration.	Acidosis.
Alkalosis.		Coma.
Dehydration.	Water.	Edema.
Thirst.		
Asthenia.	Sodium chloride.	Thirst.
		Paresis.
Hypoglycæmia.	Glucose.	Hyperglycæmia.
Collapse.		Glycosuria.
Hypocalcæmia.	Calcium.	Hypercalcæmia.
Convulsions.		Atonia, coma.

AVERAGE COMPOSITION OF HUMAN CEREBRO-SPINAL FLUID

Expressed in mg. per 100 ml.

Proteins, total :	.	.	.	10-35
Albumin, average	.	.	.	20
Globulin, average	.	.	.	3
A : G ratio	.	.	.	8 : 1
Glucose	.	.	.	45-100
Chloride, as NaCl	.	.	.	700-760
Urea	.	.	.	10- 40

AVERAGE COMPOSITION OF HUMAN BLOOD

Major constituents, expressed as percentage

	Blood, Entire.	Plasma, or Serum.
Solids, total . . .	18-25	8.5-10
Corpuscles, volume . . .	36-51	—
Proteins, total . . .	17.8-24.6	5.8-8.6
Hæmoglobin (men) . . .	13-17.5	0
(women) . . .	12-14	0
Orosins (F + A + G) : . . .	—	5.8-8.6
Fibrinogen (F) . . .	0.1-0.2	0.2-0.4
Albumin (A) . . .	—	3.4-6.7 (average, 4)
Globulin (G) . . .	—	1.2-2.9 (average, 2)
A : G ratio . . .	—	4 : 1-1.2 : 1 (average, 2 : 1)
Lipides, total . . .	0.2-2	0.45-1.2
Fatty acids . . .	0.29-0.42	0.19-0.64
Chloride, as NaCl . . .	0.45-0.53	0.56-0.62
as Cl ⁻ . . .	0.28-0.32	0.34-0.38

AVERAGE COMPOSITION OF HUMAN BLOOD

Minor constituents, expressed in mg. per 100 ml.

	Blood, Entire.	Plasma, or Serum.
Nitrogen, non-protein :	25-50	18-30
Amino acid N . . .	3-8	4-6.5
Urea N . . .	7-20	7-20
Creatine N . . .	0.6-2.9	0.2-1
Creatinine N . . .	0.3-0.7	0.3-0.7
Uric acid N . . .	0.1-1.3	0.1-1.3
Residual N . . .	3-19	2-12
Sugar, as glucose . . .	60-180	60-180
Cholesterol, total . . .	100-200	100-220
Bile acids :		
as glycocholic acid . . .	2.5-6.0	—
as Na glycocholate . . .	—	5-12
Bilirubin . . .	—	0.1-0.5
Urobilin . . .	—	0.0-0.4
Glutathione, total . . .	28-522	0.0
Lactic acid . . .	5-35	—
Creatine . . .	2-9	0.5-3
Creatinine (?) . . .	0.7-2	0.7-2
Urea . . .	15-40	15-40
Uric acid . . .	0.3-4	0.3-4
Phenols . . .	2-8	—
Indoxyl sulphate . . .	—	0.03-0.08
Ketones, as acetone . . .	1-3	—
Bromide, as NaBr . . .	—	0.5-2.5
as Br ⁻ . . .	0.23-0.57	—
Calcium, total . . .	5-7	9-11
diffusible . . .	—	3.6-4.6
non-diffusible . . .	—	2.8-6.1
Iodine . . .	0.005-0.02	—
Iron . . .	45-55	0.06-0.22
Magnesium . . .	2-4	1-4
Phosphorus, total P . . .	28-48	6-18
Inorganic P . . .	2-5	2-5
Ester P . . .	14-29	0-4
Lipoid . . .	8-18	3-14
Potassium . . .	150-250	18-21
Sodium . . .	170-225	325-350
Sulphur, non-protein :		
Total S . . .	3.8-5.1	3.1-3.9
Inorganic S . . .	0.28-0.65	0.5-1.1
Ester S . . .	0.07-0.96	0.09-0.96

Blood Analysis

Blood analysis has now become an important part of clinical medicine, both in establishing diagnoses and in observing progress of many diseases, of which diabetes and nephritis are the most obvious. By the use of a micro-technique, routine analyses can be carried out on 0.2 ml. samples of material. The methods adopted

depend on laboratory facilities and clinical requirements, and are modified in accordance with the needs of different workers. Established methods are described in the standard text-books, such as :—

Peters and Van Slyke, "Quantitative Clinical Chemistry," Vol. II.
Hawk and Bergeim, "Practical Physiological Chemistry."
Harrison, "Chemical Methods in Clinical Medicine."
Beaumont and Dodds, "Recent Advances in Medicine."

Current analytical procedure is being continually revised and augmented by work published in the scientific and medical journals. A very compact and convenient system is that of King, Haslewood and Delory, "Micro-chemical methods of blood analysis" (*Lancet*, 1937, **234**, 886 ; **235**, 346), from which, with permission of Dr. E. J. King, the following descriptions have been taken as representative of three useful types of analyses.

Collection of Material.—Capillary blood may be drawn from the ear lobe or the finger, usually the thumb above the bed of the nail. The region is cleaned with ether or alcohol, and a stab, 1–2 mm. deep, is made with a sterile puncture-apparatus or Hagedorn needle.

A 0.2 ml. pipette is filled exactly to the 0.2 ml. mark while held horizontally with its tip in the issuing blood. The pipette is then wiped free from blood, and its contents immediately discharged into the receiving tube containing saline or water.

(1) Blood Urea

Principle.—The urea is converted into ammonia by incubating the blood with urease. After removal of the proteins by zinc hydroxide, the colour given by the ammonia with Nessler's reagent is compared in a colorimeter with the colour given by a standard ammonia solution under similar conditions. By collecting the blood in an isotonic saline, the corpuscles are kept intact during incubation. Otherwise, the arginase in the red cells is liable to act on the arginine that occurs in many urease preparations, and give additional urea. Furthermore, the blood cells contain thiol compounds, notably glutathione and thioneine, that may form cloudy precipitates with the mercury in the Nessler reagent, and interfere with the colorimetry. This is also avoided by using intact blood as distinct from "laked" blood, which would be formed by osmotic rupture of the cells if the blood were diluted with water.

Blood for urea estimation may be preserved from coagulation by addition of oxalate or citrate. Fluoride must not be used as it has an inhibiting action on the urease.

Method.—(1) Transfer exactly 0.2 ml. of blood to a 15 ml. conical centrifuge tube containing 3.2 ml. of isotonic (3.0 per cent.) crystalline sodium sulphate. Rinse out the pipette three times with the sulphate, returning the rinsings to the tube.

(2) Add approximately 20 mg. of urease powder by means of the 20 mg. scoop attached to the urease bottle. Close the tube with a rubber stopper, and mix the contents gently.

(3) Incubate the tube at 37° C. for at least twenty minutes.

(4) Add 0.3 ml. of zinc sulphate and 0.3 ml. of N/2 sodium hydroxide. Mix carefully, and centrifuge the tube for five to ten minutes, until the precipitated proteins have separated out.

(5) Transfer 2 ml. of the clear supernatant liquid, which represents 0.1 ml. of the original blood, to a clean test tube. Add 5 ml. of distilled (or ammonia-free) water and 1 ml. of Nessler's reagent. Mix gently.

(6) Prepare in two separate tubes ammonia standards of (i.) low and (ii.) high concentration, respectively. The low standard is made by mixing 2 ml. of the stock ammonium chloride solution (1 ml. of which represents 0.01 mg. of ammonia nitrogen), 5 ml. of distilled water, and 1 ml. of Nessler reagent. The high standard is made by mixing 5 ml. of ammonium chloride solution, 2 ml. of water and 1 ml. of Nessler's reagent.

(7) Adjust the micro-colorimeter so that the field is uniformly lit. Half fill the right-hand cup with the low ammonia standard. Half fill the left-hand cup with the test solution. Set the standard cup at a suitable mid-way reading, say 10 or 20, on the colorimeter scale. Raise or lower the left-hand cup containing the test solution until the colours match. Take the readings of both scales. Repeat several times with different scale settings for the standard cup. If the test solution cannot be matched satisfactorily by the low ammonia standard, replace the contents of the right-hand cup by the high standard, and repeat the rest of the procedure.

(8) *Calculation.*—Since 1 mg. of nitrogen represents 2.14 mg. of urea

Low Standard

$$U = \frac{S}{\pi} \times 0.02 \times \frac{100}{\pi} \times 2.14 = \frac{S}{\pi} \times 42.8,$$

High Standard

$$U = \frac{S}{T} \times 0.05 \times \frac{100}{0.1} \times 2.14 = \frac{S}{T} \quad 107,$$

where U = urea in mg. per 100 ml. of blood,

S = scale reading of standard,

T = scale reading of test solution.

The preparation of the reagents is described in the Appendix. The normal range for blood urea is 15–40 mg. per 100 ml., representing about one-third to half of the total non-protein nitrogen. Hyperuræmia is associated with impaired renal function, particularly in chronic nephritis, and in some forms of acute nephritis, and prostatic obstruction.

(2) Non-Protein Nitrogen

The normal range of non-protein nitrogen in blood is 25–50 mg. per 100 ml. of blood. It includes: urea nitrogen, 7–20; amino acid nitrogen, 3–8; uric acid nitrogen, 0.1–1.3; creatinine nitrogen, 0.3–1.0; and residual nitrogen (including glutathione, thioneine, creatine), 3–19 mg. per 100 ml.

Principle.—The proteins of laked blood or plasma are precipitated by trichloroacetic acid, and part of the filtrate is boiled with sulphuric acid until all the nitrogenous material is converted into ammonium sulphate (Kjeldahl's reaction). The ammonia is estimated colorimetrically by Nessler's reagent.

Method.—(1) Transfer by pipette exactly 0.2 ml. of blood or plasma to a tube containing 3.2 ml. of distilled water. Rinse out the pipette three times with the water.

(2) Add 0.6 ml. of 25 per cent. trichloroacetic acid to precipitate the proteins. Close the tube with a rubber stopper and shake thoroughly. After five minutes filter the contents through a small filter into a clean tube.

(3) Add 0.5 ml. of 30 per cent. sulphuric acid to 1 ml. of the filtrate (which represents 0.05 ml. of the original blood or plasma) in a test tube, and very carefully evaporate over a small flame until the liquid darkens and white fumes are evolved. Allow the tube to cool. Add 1 drop of concentrated hydrogen peroxide (99–100 vol.) to oxidise the coloured products. Boil gently for four minutes to obtain a colourless solution. Cool, and add 5 ml. of water and 3 ml. of Nessler's reagent.

(4) Compare the colour obtained with that given by the low or the high ammonia standards, as described in the estimation of blood urea.

The excess of Nessler's reagent is added to the blood filtrate in order to neutralise the sulphuric acid, and ensure an alkaline mixture for colour development.

(5) Calculation.

Low Standard

$$N = \frac{S}{T} \times 0.02 \times \frac{100}{0.05} = \frac{S}{T} \times 40,$$

$$N = \frac{S}{T} \times \frac{100}{0.05} = \frac{S}{T} \times 100,$$

where N = concentration of non-protein nitrogen in mg. per 100 ml. of blood,

S = colorimeter reading of standard solution,

T = colorimeter reading of test solution.

When the reagents are being prepared, it is necessary to determine their ammonia value by means of a control test in which the blood is replaced by 0.2 ml. of water. The sulphuric acid may contain nitrogenous contaminants, and stabilisers containing nitrogen are often added to solutions of hydrogen peroxide. Where the reagents have a significant nitrogen value, this must be subtracted from the figure obtained for the non-protein nitrogen.

(3) Blood Sugar

The glucose value of blood in the fasting condition is 60–90 mg. per 100 ml. The "sugar value" of blood is due to glucose and traces of other saccharides, chiefly in the plasma, and nitrogenous compounds, notably glutathione, in the corpuscles. Consequently, the results obtained by many methods of blood sugar analysis really represent the total reducing substances present.

Principle (Harding, 1933).—By mixing the blood with isotonic sodium sulphate previous to precipitation by tungstate, all the corpuscles are kept intact, and the glucose in the filtrate is estimated by boiling with a copper reagent. The mixture is treated with potassium iodide, which reacts with the unreduced copper, setting free an equivalent amount of iodine that can be estimated by titration with thiosulphate.

Method.—(1) Transfer exactly 0.2 ml. of blood to a 15 ml. centrifuge tube containing 3.2 ml. of isotonic sodium sulphate. Allow the mixture to remain for four minutes to ensure diffusion of glucose from the corpuscles. Add 0.3 ml. of 10 per cent. sodium tungstate and 0.3 ml. of 7 per cent. copper sulphate ($\text{Cu SO}_4 \cdot 5\text{H}_2\text{O}$). Mix well, and centrifuge for five to ten minutes.

(2) Transfer 2 ml. of the super clear supernatant liquid, which represents 0.1 ml. of blood, to a wide ($\frac{3}{4}$ in.) test tube. Add 2 ml. of the mixed copper reagent. At the same time, prepare a control tube (or "blank") containing 2 ml. of distilled water and 2 ml. of copper reagent.

(3) Close both tubes lightly with plugs of cotton wool, and immerse in boiling water for exactly ten minutes.

(4) Cool the tubes rapidly under a water tap. To each add 2 ml. of 1 per cent. potassium iodide and 2 ml. of N. sulphuric acid.

(5) After standing for one minute the contents of each tube are titrated with 0.005N (N/200) sodium thiosulphate, a few drops of 1 per cent. soluble starch (in water or in a saturated solution of phenol red) being used as indicator. The end-point is shown by the complete disappearance of the blue colour of the starch iodine complex.

(6) *Calculation.*—The thiosulphate value for the sugar is obtained by subtracting the titration figure for the test solution from that obtained for the control solution.

Then, since 1 ml. 0.005 N. thiosulphate represents 0.116 mg. glucose,

T (the thiosulphate value) $\times 0.116 =$ glucose, in mg. per 2 ml. of filtrate, or in 0.1 ml. of blood.

Glucose, in mg. per 100 ml. blood $= T \times 0.116 \times \frac{100}{0.1} = T \times 116$,

where $T =$ (titration value for control — titration value for test solution).

Blood sugar is rapidly destroyed by the enzymes in the blood; and specimens kept for subsequent analysis should have a little powdered fluoride and thymol added.

The Conway Micro-diffusion Unit. This apparatus consists of a pyrex glass container, within which is an inner compartment formed by a circular wall of glass, about half the height of the outer wall. The unit is covered by a square glass plate and made air-tight by soft paraffin.

The volatile material to be estimated is placed in the outer annular compartment, the absorbent being in the inner compartment, where it is titrated subsequently without removal from the unit. The apparatus may be employed for the micro-estimation of ammonia, urea, chloride, bromide and carbonate, and for the detection of many volatile substances, such as alcohol, aldehydes and acetone. For details of the apparatus and its applications consult Conway, E. J. (1939), "Micro-Diffusion Analysis." The various micro-methods available for the clinical estimation of urea are critically discussed by Lee and Widdowson (*Biochem. J.*, 1937, **31**, 2035). The error arising from formation of ammonia by an enzyme-substrate system in jack bean urease extracts has been studied by Howell (1939), who reports that it can be eliminated by the use of extracts prepared five to twenty hours before use, and by keeping the pH value of the digests between 6 and 6.6 by means of a citrate buffer.

(4) Blood Pigments

Hæmoglobin. According to Van Slyke, the best method for expressing the hæmoglobin value of blood is in terms of the oxygen-

combining power, on the assumption that 1 gm. of hæmoglobin can unite with 1.34 ml. of oxygen. The hæmoglobin value may also be expressed in terms of the iron content of the blood, since hæmoglobin contains 0.0335 per cent. of iron. These methods, however, are outside the scope of rapid clinical work, although they may be used to calibrate the colorimetric methods employed clinically.

Colorimetric methods depend on the conversion of hæmoglobin into a suitable derivative, carboxyhæmoglobin or acid hæmatin, and subsequent comparison with an appropriate standard. Many different types of hæmoglobinometer are in use, and with the better instruments the error should not exceed ± 5 per cent.

The simplest way to express the result is in terms of grams of hæmoglobin per 100 ml. of blood. It also may be expressed as a percentage of the average hæmoglobin value of human blood. This was originally stated by Haldane to be 14 gm. hæmoglobin per 100 ml., but a range of 12 gm. to 17.5 gm. has been recorded for normal adult subjects, depending on the concentration of the red corpuscles. J. McGrath defines the standard for hæmoglobin as the percentage present in the blood of a normal adult with 5,000,000 red cells per c.mm.

Methæmoglobin and Sulphæmoglobin. These abnormal pigments may appear in the blood in some infective diseases, or as the result of administration of certain benzene derivatives, among which drugs of the sulphonamide group are probably the most important. The onset of the condition is marked by a cyanosis, which in methæmoglobinæmia is of short duration, but when due to sulphæmoglobinæmia may persist for several weeks.

Although the pigments may be identified spectroscopically, the observation is made difficult by the fact that the characteristic absorption bands of the two pigments lie in the same vicinity at the red end of the spectrum, and may be obscured by the presence of oxyhæmoglobin, if the solution is too concentrated. According to Campbell and Morgan (1939), the samples of blood (2 ml.) should be laked with not more than 4 ml. of water, and examined spectroscopically immediately after withdrawal.

The methæmoglobin band extends from 625 $m\mu$ to 655 $m\mu$.

The sulphæmoglobin band extends from 615 $m\mu$ to 630 $m\mu$.

Preparation of a Methæmoglobin Solution for Comparison. To 5 ml. of blood, diluted 1 in 50, add 2 drops of a saturated solution of potassium ferricyanide. Shake the mixture and examine after a few minutes. At this dilution, usually one band in the red (625 $m\mu$ to 630 $m\mu$) is visible.

Dilute gradually with an equal volume of water and observe

changes in the spectrum. The band at $625\text{ m}\mu$ persists, while faint bands at $540\text{ m}\mu$ and at $575\text{ m}\mu$ may become visible.

Make the mixture alkaline by addition of 5–10 drops of 1 per cent. sodium carbonate. Observe the spectrum, which now shows the band at $600\text{ m}\mu$ to $610\text{ m}\mu$ that is characteristic of alkaline methæmoglobin. The band in the region $625\text{ m}\mu$ is no longer visible, although the bands at $540\text{ m}\mu$ and $575\text{ m}\mu$ may persist.

Sulphæmoglobin, HbSH_2 , is formed by the action of reducing agents on hæmoglobin in presence of H_2S , and for this reason sulphates or compounds liable to give rise to H_2S should not be administered along with sulphonamide or similar drugs.

APPENDIX

REAGENTS EMPLOYED BIOCHEMISTRY

(In aqueous solution, unless otherwise stated.)

Antimony trichloride, 30 per cent. in chloroform. Reagent for vitamin A and other carotinoids (p. 204).

Benedict's Qualitative Copper Reagent: 17.3 gm. crystalline $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, with 173 gm. Na citrate and 100 gm. anhydrous Na_2CO_3 in 1,000 ml. water. Used for detecting reducing sugars in urine.

Benedict's Quantitative Copper Reagent: 18.0 gm. crystalline $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, with 200 gm. Na citrate, 125 gm. KSCN, and 5 ml. of 5 per cent. K ferrocyanide, made up to 1,000 ml. with water. Before use, add 3-5 gm. anhydrous Na_2CO_3 to 25 ml. of reagent. Used for estimating reducing sugars.

Benzidine, fresh saturated solution in glacial acetic acid. Reagent for blood pigment in urine.

Diazo-Reagent (Ehrlich, Van den Bergh), freshly prepared mixture of 1 ml. of 0.5 per cent. NaNO_2 and 50 ml. of 0.1 per cent. sulphanilic acid in 2 per cent. HCl. Reagent (in acid solution) for free bilirubin; and (in alkaline solution) for histidine, histamine, tyrosine, tyramine, indoxyl, and polyphenols.

3, 5-Dinitrobenzoic Acid, 5 per cent. Yields a purple colour with creatinine in alkaline solution. The reagent may be used in alcoholic solution, or dissolved in dilute sodium carbonate.

Ehrlich's Aldehyde Reagent, 2-3 per cent. *p*-dimethylamino benzaldehyde in alcohol or in 20 per cent. HCl. Reagent for mucoproteins, indole, indoxyl, urobilinogen, urea, allantoin; and (in excess of strong acid) tryptophane and scatole. The reagent in 20 per cent. HCl is almost colourless, and is suitable for the urea and allantoin tests.

Fehling's Reagent. See p. 111.

Folin's Reagent, for uric acid and phenols. Boil under a reflux condenser for two hours a mixture of : 100 gm. sodium tungstate, 102 ml. *ortho*-phosphoric acid (B.P. 66.3 per cent.), and 750 ml. of water. When the mixture has cooled, sufficient bromine is added to bleach completely any blue colour. The mixture is then boiled

to remove the excess of bromine, cooled and made up to 1 litre with water.

Guaiacum, 2 per cent. in alcohol. With H_2O_2 , it is a reagent for peroxidases, and hæmoglobin in urine. With acetic acid, it is a reagent for nitrite in saliva.

Glyoxylic Acid Reagent. Add slowly 4 gm. of powdered Mg. to 100 ml. saturated oxalic acid. Filter when reduction is complete, and dilute with water to 400 ml. Used as a ring test with H_2SO_4 for the detection of tryptophane in proteins. Also gives colours with indole and scatole.

H-ion Indicators. These reagents change colour sharply at a particular concentration of H-ions. A large number are in practical use, covering the acid-alkali scale from pH 0.1 to about pH 13. For details of application consult: *Practical Physiological Chemistry*, by S. W. Cole; *H-ion Concentration*, by F. L. LaMotte, W. R. Kenny, and A. B. Reed; *Quantitative Inorganic Analysis*, by A. I. Vogel.

H-ion Indicators

Indicator.	pH Range ← Acidity—Alkalinity →
Methyl violet . . .	yellow 0.1—1.5 green
Thymol blue . . .	red 1.4—2.8 yellow
Methyl violet . . .	green 1.5—3.2 violet
Tropæolin OO . . .	pink 1.5—3.0 yellow
Methyl yellow . . .	red 2.9—4.0 yellow
Methyl orange . . .	orange 3.0—4.4 yellow
Tetrabromphenol blue . .	yellow 3.0—4.6 blue
Bromcresol green . . .	yellow 3.8—5.4 blue
Methyl red . . .	red 4.3—6.2 yellow
Litmus . . .	red 5.0—8.0 blue
Tashiro's indicator . . .	pink 5.45—5.50 green
Bromthymol blue . . .	yellow 6.0—7.6 blue
Phenol red . . .	yellow 6.7—8.3 red
Thymol blue . . .	yellow 8.0—9.6 blue
Phenolphthalein . . .	colourless 8.2—10.0 red

These indicators are generally used in 0.04 per cent. aqueous solution, with the exception of phenolphthalein, which is dissolved in alcohol.

For gastric analysis and titration stronger solutions (1 per cent.) of methyl violet, thymol blue, and methyl yellow are used.

Methyl yellow is also known as Töpfer's indicator or dimethyl-amino-azo-benzene. It will be seen that some of the indicators have an

extended pH range in that they show more than two colours as the reaction changes from acidity to alkalinity. Examples of these indicators are methyl violet, which changes from yellow, green, blue, to violet, and thymol blue, which changes from red, yellow, green, to blue.

This property is developed in the use of mixed reagents, such as the B.D.H. "universal indicator," which has the following colour range:

pH up to 3.0	Red	pH 8.0	Green
4.0	Deeper Red	„ 8.5	Bluish Green
5.0	Orange Red	„ 9.0	Greenish Blue
5.5	Orange	„ 9.5	Blue
6.0	Orange Yellow	„ 10.0	Violet
6.0	Yellow	„ 10.5	Reddish Violet
7.0—7.5	Greenish Yellow	„ 11.0	Deeper Reddish Violet

Litmus is included in the list to show how insensitive it is, relatively, when compared with the newer reagents. A change of three pH degrees is required to alter it from red to blue. Phenol red is a much sharper indicator for titrations to the end-point of absolute neutrality, pH 7.0.

For general use, one drop of indicator is added to each millimetre of solution examined. In exact work, the resulting colour is matched with a standard in a comparator.

Tashiro's Indicator. The stock solution is made by mixing 200 ml. of 1 per cent. alcoholic methyl red with 50 ml. of 0.1 per cent. alcoholic methylene blue. Both dyes must be dissolved in pure alcohol. For use, 1 part of stock solution is mixed with 1 part of alcohol and 2 parts of distilled water. This is an example of a *screened* indicator, or one in which the colour change is made more distinct by addition of a second pigment (methylene blue) to cut out part of the spectrum. The indicator is used in the Conway methods for the micro-analysis of ammonia and urea.

Fluorescent Indicators. These reagents respond to a change in pH by changing in colour or in intensity of fluorescence when exposed to ultra-violet light, and are very useful for analysis involving darkly-coloured liquids. The solution to be titrated is placed in a thin-walled flask or beaker on a black surface screened from sunlight. During the titration the mixture is illuminated obliquely by a beam of ultra-violet light to show the change in fluorescence.¹

¹ An ultra-violet lamp suitable for use while titrating has been introduced by Messrs. Baird and Tatlock (London) Ltd., and it and the appropriate fluorescent indicators can be obtained from Messrs. Hopkin and Williams Ltd., of 16 St. Cross Street, London.

Fluorescent Indicators

Indicator.	pH Range ←Acidity—Alkalinity→	
3 : 6-Dihydroxy-phthalimide	colourless	0.0—2.5 yellow-green
	yellow-green	7.5—8.5 green
3 : 6-Dihydroxy-	colourless	0.2—1.5 blue
phthalonitrile	blue	6.0—8.0 green
Fluorescein	green	0.1—0.5 yellow
Eosin	red	2.0—3.5 yellow
Erythrosin B	red	3.0—5.0 yellow
Acridine	green	4.5—5.5 blue
Quinine sulphate . .	blue	4.5—8.5 violet
	violet	8.5—9.5 colourless
β -Naphthol	colourless	6.0—6.5 blue

Methylene Blue, 0.1 per cent. Redox indicator used in determining the end-point in Fehling's sugar estimation.

Millon's Mercury Reagent. 3 gm. Hg dissolved in 4 ml. concentrated HNO_3 , diluted to 10 ml. with water, and filtered. Used for detecting tyrosine in proteins and phenols in urine.

α -Naphthol, 2 per cent. in alcohol. General reagent for carbohydrates, also used to detect arginine in proteins and indoxyl in urine.

β -Naphthoquinone Sulphonate, 10 per cent. General reagent for proteins and amino acids.

Naphtho-resorcinol, 1 per cent. in alcohol. Special reagent for glycuronic acid in urine.

Ninhydrin, 0.2 per cent. General reagent for proteins and amino acids.

Phenylhydrazine Hydrochloride: Osazone reagent for sugars.

Potassium Chromate, 5 per cent. With concentrated HNO_3 , reagent for all primary and secondary alcohols, including sugars, glycerol, lactic acid, β -hydroxy-butyric acid, and tartaric acid.

Resorcinol, 5 per cent. in alcohol. Reagent for ketoses and indoxyl.

Schiff's Rosaniline Reagent, 1 per cent. rosaniline decolourised by SO_2 . General reagent for free aldehyde groups.

Salicylsulphonic Acid, 20 per cent. General precipitant for higher proteins.

Sodium Nitroprusside (Nitroferrocyanide), 5 per cent. Reagent for sulphhydryl compounds, including cysteine, thioneine, and glutathione. Also reacts with acetone, acetoacetic acid, creatinine and indole.

The reagent is unstable if exposed to light, and should be kept in the dark or stabilised by addition of a couple of drops of nitric acid.

Standard Sugar Solution (Lane and Eynon). Dissolve exactly 9.5 gm. of pure sucrose in 100 ml. of approximately 2 per cent. (or 0.6 Normal) HCl. The mixture is kept for a week, during which time it undergoes hydrolysis to "invert" sugar, an equimolecular mixture of glucose and fructose. It is then diluted to 1 litre and stored. For standardisation, 50 ml. is neutralised and made up to 100 ml. with water to yield a 0.5 per cent. solution of "invert" sugar.

Thymol 3 per cent. in alcohol. General reagent for carbohydrates. Also reacts with indoxyl.

Xanthidrol, 10 per cent. in methyl alcohol. Reagent for urea.

Reagents Used in Blood Analysis

Nessler's Reagent (Koch and McMeekin, 1924). Dissolve 22.5 gm. of iodine in 20 ml. of water containing 30 gm. of potassium iodide. Keeping the mixture cool by means of running water, add 30 gm. of mercury, and shake until the yellow colour has disappeared.

Decant the supernatant liquid into a 200 ml. flask, and test a drop of the solution with a drop of 1 per cent. soluble starch. If a faint blue colour does not appear, add a few drops of the original iodine solution to the decanted liquid until a faint excess of iodine can be detected by the starch test. Dilute the liquid to 200 ml., and pour into 975 ml. of 10 per cent. sodium hydroxide. Mix and allow the reagent to clear by subsidence.

Ammonium Chloride Standard, containing 0.01 mg. of ammonia nitrogen per ml. Dissolve exactly 153 mg. of analytically pure ammonium dry ammonium chloride in distilled ammonia-free water, and make up to 100 ml. 25 ml. of this solution are acidified with 10 ml. of N. sulphuric acid and diluted to 1 litre with ammonia-free water.

Sodium Sulphate, isotonic. Dissolve 30 gm. of crystalline sodium sulphate ($\text{Na}_2\text{SO}_4 \cdot 10 \text{H}_2\text{O}$) in water, and make up to 1 litre.

Zinc Sulphate. Dissolve 10 gm. of crystalline zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) in water, and make up to 100 ml.

Sulphuric Acid, 30 per cent., by volume. Add slowly 30 ml. of analytically-pure sulphuric acid to about 60 ml. of distilled water in a 100 ml. flask. Mix, cool to room temperature, and make up to 100 ml.

Copper Reagent (Harding, 1932). A. Dissolve 13 gm. of pure crystalline copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), in water, and make up to 1 litre.

B. Dissolve *separately* in minimum quantities of water at room temperature each of the following: 24 gm. sodium potassium tartrate, 40 gm. anhydrous sodium carbonate, 50 gm. sodium hydrogen carbonate, 36.8 gm. potassium oxalate, and *exactly* 1.4 gm. potassium iodate.

Mix the solutions and make up to 1 litre.

The copper reagent is a freshly-prepared mixture of *exactly equal* volumes of A and B.

The materials used should be of analytical purity, and each new batch of reagent should be checked against a solution of pure sugar.

ERRATA

Page 18, line 31, and page 101, line 27: *for* phytol *read* phytin.

Page 30, line 8 from foot: *for* $\text{H}_4\text{P}_2\text{O}_8$ *read* $\text{H}_4\text{P}_2\text{O}_7$.

Page 57, line 16 and line 17: *for* anions *read* cations.

Page 57, line 18: *for* cations *read* anions.

Page 59, line 32 and page 61, line 5: *for* Cl^+ *read* Cl^- .

Page 189, line 19: *for* hæmhæm *read* hæm.

Page 206, line 12: *for* co-carboxylase *read* co-deaminase.

Page 248, line 25: *for* gm. *read* mg.

Page 251, line 2 from foot: *for* -ethyl- *read* -methyl-.

Page 287, line 21 and line 22: *for* 648 *read* 756

Page 360, line 12: formula should be $\text{H}_2\text{N.C}(\text{:NH}).\text{N}(\text{CH}_3).\text{CH}_2.\text{COOH}$.

Page 373, last line: *for* triphosphate *read* pyrophosphate.

Page 436: in the structural formula for Progesterone and for Pregnandiol the side-chain should be attached to the apex of ring IV, at C 17 (*cf.* p. 172).

INDEX

*'index-learning turns no student pale,
Yet holds the eel of science by the tail.*

(ALEXANDER POPE)

- Acetaldehyde, 299, 321
- Acetic acid, derivatives, 136
 - series, 160
- Acetic fermentation, 298
- Acetoacetic acid, 286, 319, 407
 - in urine, 407
- Acetone, 286, 319
 - in urine, 408
- Acetonitrile test, 419
- Acetyl choline, 363, 414, 443
- Acetyl glucosamine, 98
- Achroodextrin, 94
- Acids, definition, 49, 52
 - estimation, 271, 393
- Acrolein, 163
- Acromegaly, 440
- Actinoerythrin, 204
- Activators, catalyst, 214
- Addison's disease, 415
- Adenase, 221, 350
- Adenine, 130, 290, 344
- Adenosine, 306, 344, 347, 349
 - triphosphate, 290
- Adenylic acid, 290, 291, 306, 348, 444
- Adermine, 257, 258
- Adipic acid, 105
- Adrenal hormones, 182, 414
- Adrenaline, 300, 308, 366, 416
- Adrenaline oxidase, 223
- Adrenergic fibres, 365
- Adrenine. *See* Adrenaline
- Adrenosterone, 415, 434
- Adrenotropic principle, 437
- Adsorption, 67, 229
- Aerobic glycolysis, 289
 - organisms, 32
 - oxidation, 289, 338
- Ætiophyllin, 196
- Ætioporphyrin, 187
- Agar-agar, 97
- Aglucone, 78, 98
- Agmatine, 360
- Agnosterol, 173
- Alanine, 137, 142, 442, 445
 - metabolism, 307
- Albinism, 226, 315, 409
- Albuminoids, 126
- Albumins, 125, 127, 154
- Albuminuria, 401
- Alcaptonuria, 315, 408
- Alcohol, tests, 109, 408
 - dehydrogenase, 329
- Alcoholic fermentation, 298
- Aldehyde mutase, 223
- Aldehyde oxidase, 224
- Aldenol configuration, 84
- Aldose sugars, 76, 83
- Alimentary hormones, 441
- Aliphatic acids, 160
- Aliphatic alcohols, 162
- Alkaline-earth metals, 17
- Alkaline metals, 11
- Alkaline tide, 393
- Allantoin, 346, 397
- Allergy, 280
- Alloxan, 346
 - test, 143
- Alloxazine, 206
- Allyl sulphide, 33
- Aloin, 80
- Aluminium, 24
- Aminases, 215, 221
- Amines, 143, 356
 - depressor, 357
 - pressor, 356
 - proteinogenous, 357
- Amine oxidase, 223
- Amino acids, 133, 135-151, 303
 - deamination, 304
 - distribution, 144
 - essential, 314
 - glucogenic, 141, 283
 - ketogenic, 141
 - reactions, 143
 - tests, 148-150
 - transamination, 303
- Aminolipides, 159
- Aminopherase, 304
- Aminopurines, 344
- Aminosaccharides, 78, 99
- Ammonia, 29, 133, 373
 - in urine, 393
- Ammonia carriers, 306
- Ammoniacal fermentation, 298, 376

- Amphotericity**, 136
Amylase, 213, 217
Amylopectin, 93
Amylose, 93
Amylum, 92
Anæmia, 263
Anaerobic glycolysis, 288
Anaerobic organisms, 33
Androgens, 180, 182, 434
Androstandiol, 435
Androstane, 182, 435
Androsterone, 182, 415, 434, 436
Aneurin, 252
Angioxyl, 444
Anserine, 314, 374
Anthocyanin, 99
Anthracene, 74
Anti-diurein, 438
Anti-enzymes, 214
Apo-zyrnase, 298
Araban, 78, 80, 91, 97
Arabinosazone, 115
Arabinose, 77, 80
Arachidic acid, 162
Arginase, 41, 222, 386
Arginine, 138, 312, 360, 371, 386
 test, 148
Arsenic, 32
Aschheim-Zondek test, 434
Ascorbic acid, 259-263
Ascorbic oxidase, 260
Asparaginase, 221
Asparagine, 139, 387
Aspartic acid, 139, 304, 306
Atomic weights, 6
Atropine, 357, 366
Autacoids, 412
Autolysis, 211
Autotrophes, 72, 234
Auxins, 445
Azelaic acid, 161
- Barium**, 22
Bases, 49
Batyl alcohol, 163
Beeswax, 167
Beet Sugar. *See* Sucrose.
Benedict's reagents, 110, 112, 121
 test, in urine, 405
Benzanthrene, 182
Benzedrine, 374
Benzidine test, 403
Benzpyrrole, 310
Beri-beri, 253
Betaines, 356, 362
Bial's test, 112
Bile, 274-278
 acids, 177, 277
 pigments, 275, 404
 salts, 274, 405
- Bilipurpurin**, 184
Bilirubin, 185, 199, 275
Biliuria, 404
Biliverdin, 185, 275
Biological elements, table, !
 Group I., 11
 Group II., 17
 Group III., 24
 Group IV., 25
 Group V., 28
 Group VI., 32
 Group VII., 35
 Group VIII., 38
Bionic acid, 105
Bios, 445
Biosphere, 43
Biuret, 382
 test, 148
Blood, analysis, 451
 composition, 450
 detection, 403
 sugar, 283, 285, 455
 urea, 452
Bone, calcium, 22
 magnesium, 18
Boric acid, 107
Boron, 24
Bradycardia, 254
Brain, metabolism, 294
Bregenin, 159
Bromelin, 220
Bromine, 37
Buffer systems, 53
Bufotoxin, 179
Bush sickness, 41
Butter, 165
Butyric acid, 138, 272, 321
Butyrine, 138
- Cacao butter**, 165
Cachexia strumipriva, 420
Cadaverine, 359
Cadmium, 17
Calciferol, 180, 246
Calciovarin, 247
Calcium, 19-22
 nutritional, 236
 in urine, 390
Canaline, 313
Canavanine, 138, 313
Cane sugar. *See* Sucrose.
Cannizzaro reaction, 104
Capric acid, 160
Caproic acid, 139, 160
Caprylic acid, 160
Caramel, 82
Carbamate, 55
Carbamide. *See* Urea.
Carbohydrases, 215
Carbohydrates, 76-101, 236

- Carbohydrates**, circulation, 288
 classification, 77
 metabolism, 282-302
 reactions, 102-124
Carboligase, 224
Carbon, 25-27, 55
Carbon dioxide, 55
Carbonic anhydrase, 56
Carboxyhæmoglobin, 191, 195
Carboxylase, 143, 228, 298
Carcinogens, 182
Cardiac metabolism, 293
Cardio-toxic glucosides, 179
Carnauhyl alcohol, 162
Carnosine, 314, 374
Carotene, 160, 201, 241, 323
Carotinoids, 200-205
Carriers, respiratory, 330, 338
Casein, 129
Caseinogen, 129, 153
Catalase, 39, 227, 328, 330
Catalysis, 209
Cellobiase, 217
Cellobiose, 88
Cellulase, 217
Cellulose, 91, 97
Cephalin, 159, 167
Cerebronic acid, 162
Cerebrosides, 159
Cerebro-spinal fluid, 450
Cerotic acid, 160
Cetyl alcohol, 162
Chaulmoogric acid, 162
Chitin, 98
Chitosamine, 99, 133
Chloride, estimation, 394
 shift, 37, 58
Chlorine, 36
Chloroæruorin, 39, 131, 189
Chlorophyll, 196
Chlorophyllase, 196, 216
Cholecystokinin, 443
Cholehæmatin, 198
Cholestane, 172
Cholesterol, 173-176, 275, 323
Cholic acid, 177, 275
Choline, 168, 264, 356, 362
 nitrite, 363
Cholinergic fibres, 365
Chondroitin, 98
Chondrosamine, 99, 133
Chromatin, 130
Chromatographic analysis, 201
Chromium, 32
Chromoproteins, 125, 133
Chromotropic factor, 439
Chymotrypsin, 229
Citric cycle, 334
Citric dehydrogenase, 329
Citrin, 240
Citrulline, 138, 313, 386
Citrulline test, 150
Clupadonic acid, 162
Coagulation, 68, 132, 151
Cobalt, 41
Co-carboxylase, 206, 254, 331, 336
Co-dehydrogenases. *See* Co-enzymes
Co-enzyme I., 214, 259, 331, 333
Co-enzyme II., 214, 259, 296, 331, 333
Colamine, 168, 362
Collagen, 127
Colloidal systems, 64-71
Contraction process, 289
Copper, 15
 protein test, 148, 384
 reduction test, 110
Coproporphyrin, 187
Coprostanol, 173
Coprosterol, 174, 275
Cori cycle, 287
Cori ester, 100, 292
Cortin, 182, 415
Co-zyrmase. *See* Co-enzyme I.
Creatine, 136, 360, 367-373
Creatine phosphate, 290
Creatinine, 368
Creatinuria, 368
Cresols, 399, 400
Cretinism, 420
Cromaffin tissue, 418
Cyanic acid, 382
Cyclol theory, 147
Cyclopeptides, 147
Cycloses, 100
Cysteic acid, 178
Cysteine, 137, 178, 310
Cystine, 137, 150, 310
Cystinuria, 315, 409
Cytochrome, 40, 131, 188, 195, 226
 dehydrogenase, 226, 330
Cytosine, 130, 353
Dahlia starch, 96
Deaminases, 224
Deamination, 141, 304, 382
Decarboxylation, 143
Dehydroandrosterone, 182, 434
Dehydroascorbic acid, 260
Dehydrobilirubin, 184
Dehydrocholesterol, 173
Dehydrogenases, 215, 224, 328, 337
Denaturation, 125, 132
Depressor amines, 357
Desaturation, 318
Desmo-enzymes, 210
Desmolases, 215, 228
Desoxycholic acid, 177
Desoxy-sugars, 100
Detoxification, 105, 406
Deuterium, 11
Dextrin, 94, 97

- Dextrinose, 86
 Dextrose, 81
 Diabetes insipidus, 438
 Diabetes mellitus, 284, 301, 424
 Diabetogenic factor, 427
 Diacetyl test, 150, 384
 Diaphorase, 224
 Diastase, 211, 217
 Diazo test, for bilirubin, 277
 for histamine, 358
 Dietary standards, 265
 Digestion, alimentary, 266-281
 summary, 279
 Digitalis, 179
 Digitonin, 179
 Dihydroxyacetone, 77
 Di-iodo-tyrosine, 137
 Diose, 77
 Dipeptidase, 221
 Dipeptide, 145
 Dipolar ion, 52, 136
 Disaccharidase, 218
 Disaccharides, 77, 119
 Dissociation constant, 49
 Donnan membrane effect, 58
 Dopa, 418
 Dopa oxidase, 309, 330
 Drying-oils, 162, 168
- Edestin, 128
 Egg proteins, 127, 129
 Ehrlich's aldehyde reaction, 149, 459
 allantoin, 347
 indole, 310
 tryptophane, 149
 urea, 397
 urobilinogen, 393
 Ehrlich's diazo reaction, 459
 bilirubin, 277
 Elastin, 127
 Electrode potential, 341
 Electrophoresis, 67
 Embden ester, 100
 Emmenine, 429
 Emulsin, 218
 Emulsoid, 66
 Endocrines, 412
 Endopeptidase, 218
 Enol configuration, 75
 Enterokinase, 279
 Enzymes, 211-232
 classification, 214
 definitions, 210
 mechanism, 229
 Ephedrine, 366
 Epimer, 79
 Epinephrine. *See* Adrenaline.
 Equilene, 181, 429, 436
 Equilenin, 181, 429, 436
 Erepsin, 220
- Ergosterol, 173-176, 246
 Ergothioneine, 314, 362
 Erucic acid, 162
 Erythrocrucorin, 189
 Erythrodextrin, 94
 Erythrol, 98
 Esterase, 215
 Evocators, 446
 Exopeptidase, 220
 Exelsin, 128
 External phase, 65
- Faraday-Tyndall effect, 66
 Fat, 164
 absorption, 316
 classification, 158
 hydrolysis, 165
 metabolism, 317-322
 Fat-soluble vitamins, 240
 Fatty acids, 160
 Fehling's test, 110, 406
 Female sex hormones, 427
 Fermentable sugars, 114
 Fermentation, alcoholic, 113, 297
 ammoniacal, 376
 lactic, 87, 298
 Ferroprotein, 331
 Feulgen's test, 130, 349
 Fibrinogen, 188, 450
 Fibrous proteins, 145
 Flavins, 205
 Flavoproteins, 131, 206, 332, 333
 Flavopurine, 206
 Flavoxanthin, 201
 Fluorine, 35
 Follicle stimulating hormone (FSH), 430
 Foodstuffs, 234-265
 classification, 234
 requirements, 264
 Formaldehyde, 77
 Formic acid, 160
 Fractional precipitation, 151
 Free water, 46
 Friedman test, 434
 Fröhlich syndrome, 440
 Fructosan, 78, 96
 Fructose, 82, 90
 Fructose diphosphate, 292
 monophosphate, 99
 Fruit sugar. *See* Fructose.
 Fumarase, 328, 334
 Fumaric acid, 328, 334
 Furan, 74, 85
 Furanose, 84
 Furfuraldehyde (furfural), 85, 102, 112
- Galactosazone, 115, 117
 Galactose, 82, 114
 Galactoside. *See* Glycolipide.

- Galactosides, 159
 Galacturonic acid, 99
 Galegine, 360
 Gastric digestion, 268-273
 juice, 268
 Gastrin, 359, 441
 Gelatin, 137
 Genin, 179
 Gentianose, 86
 Gentiobiose, 86, 88
 Germanium, 38
 Gigantism, 440
 Glass electrode, 50
 Gliadin, 125, 126
 Globin, 126, 131
 Globular proteins, 145
 Globulins, 125, 128, 154
 Glucides, 76
 Glucogenic amino acids, 141
 Gluco-kinetic hormones, 300
 Gluconic acid, 83
 Glucosamine, 99, 133
 Glucosans, 78, 91
 Glucosazone, 106, 115, 116
 Glucose, 81, 83
 dehydrogenase, 329
 metabolism, 288
 monophosphate, 100
 structure, 83
 tests, 110
 threshold, 284
 tolerance, 284
 Glucose-lactate cycle, 286
 Glucosides, 78
 Glucuronic acid, 99, 105, 406
 Glutamic acid, 139, 304, 306, 311
 Glutaminase, 221, 312
 Glutamine, 139, 387
 Glutamine cycle, 387
 Glutaric acid, 139, 304, 310
 Glutathione, 139, 214, 296, 310, 331, 334
 Glutelins, 125
 Gluten, 126
 Glutenin, 126
 Glycals, 100
 Glycerides, 164
 Glyceroboric acid, 107
 Glycerol, 98, 159, 163, 299, 321
 Glycerophosphate dehydrogenase, 329
 Glycerophosphoric acid, 163
 Glycerose, 77
 Glycine, 136, 177, 306
 Glycocholic acid, 277, 307, 177
 Glycocyamine, 360, 371
 Glycogen, 94, 97, 283
 Glycogenase, 283, 296
 Glycogen-lactate system, 291
 Glycogenolysis, 291
 Glycol aldehyde, 77
 Glycolipides, 159, 169
 Glycollic acid, 307
 Glycoproteins, 125, 130
 Glycosides, 77, 78, 99
 Glycosuria, 284, 301, 405
 Glycotropic principle, 438
 Glycyl-glycine, 145
 Glyoxalase, 225, 296
 Goitre, 38, 420
 Göthlin's test, 263
 Gonadotropic hormones, 430, 437, 441
 Grape sugar. *See* Glucose.
 Guaiacum test, 403
 Guanase, 221, 351
 Guanidine, 148, 345, 356, 359
 Guanine, 130, 222, 344
 Guanosine, 347
 Guanylic acid, 348
 Gulonic acid, 260
 Günzberg's test, 270
 Gynæcogens, 170, 427
 Hæm, 189, 191, 193
 Hæmatin, 39, 131
 Hæmatoporphyrin, 187, 193
 Hæmaturia, 403
 Hæmin, 189, 192
 Hæmochromes, 131, 189
 Hæmochromogen, 189, 191
 Hæmocuprein, 16
 Hæmocuprin, 16
 Hæmocyanin, 16, 131, 189
 Hæmoerythrin, 39, 189
 Hæmoglobin, 39, 131, 189, 193-195
 conversion into bilirubin, 199
 Hæmopoietic factor, 263
 Harden's test, 150
 Harden-Young ester, 99, 107, 292
 Heat-coagulation, 132, 127, 151
 Heavy water, 11
 Heller's test, 151
 Heme. *See* Hæm.
 Hemeralopia, 243
 Heparin, 98
 Hepatocuprein, 16
 Hepatoflavin, 205
 Heptose, 77
 Hesperidin, 240
 Hetero-auxin, 446
 Heterohexosan, 96
 Heteropentosan, 96
 Heterosaccharide, 76, 78
 Heterotrophes, 72
 Hexosamines, 78, 97, 98, 133
 Hexosans, 78, 91
 Hexoses, 77-81
 absorption, 282
 Hexose diphosphate, 99, 292, 299
 dehydrogenase, 330
 Hexose monophosphates, 99
 dehydrogenase, 329

- Hexuronic acid, 259
 Hippuric acid, 136, 307, 399
 Hippuricase, 221
 Histamine, 143, 314, 354, 357, 441
 Histamine oxidase, 223
 Histidase, 313
 Histidine, 138, 313, 358
 Histohæmatin, 195
 Histones, 125
 Hogben's test, 434
 Holosaccharides, 91, 96
 Homogentisic acid, 307, 308, 409
 Hopkins-Cole test, 149
 Hordein, 126
 Hordenine, 366
 Hormones, 412
 Hormoproteins, 125, 131
 Hydrazones, 105
 Hydrochloric acid, gastric, 269-271
 Hydrogen, 11
 electrode, 50
 Hydrogen ion, 48-55
 Hydrogen peroxide, 227, 237
 Hydrogen transfer, 327, 333
 Hydroquinol, 399
 β -hydroxybutyric acid, 286, 319
 dehydrogenase, 329
 β -hydroxyglutamic acid, 139
 Hydroxynervone, 170
 Hydroxyproline, 140, 312
 Hydroxypurines, 345
 Hypercalcaemia, 423
 Hyperchromic anæmia, 263
 Hyperparathyroidism, 423
 Hyperpituitarism, 440
 Hyperthyroidism, 420
 Hypoglycaemia, 289, 300, 425
 Hypoparathyroidism, 423
 Hypophamine, 438
 Hypopituitarism, 439
 Hypothyroidism, 420
 Hypoxanthine, 222, 344

 Iminazole, 74, 314
 Imino acid, 133, 305
 Indican. *See* Indoxyl.
 Indicators, H-ion, 54
 redox, 342
 Indigo blue, 400
 Indole, 74, 309, 310
 Indole acetic acid, 416
 Indole pyruvic acid, 310
 Indophenol oxidase, 226
 Indoxyl, 309, 400
 Inositol, 101, 445
 Insulin, 300, 424-427
 Internal phase, 65
 Interrenalin, 415
 Intestinal absorption, 279
 Intestinal juice, 278

 Inulase, 96
 Inulin, 78, 96
 Invertase, 89, 218
 Invert sugar, 89
 Iodine, 38
 Iodine test, for bilirubin, 276, 404
 for polysaccharides, 109
 Iodoacetic acid, 238
 Iodogorgic acid, 38, 137
 Ionic systems, 47
 Ionone, 160, 203
 Iron, 38, 390
 Iso-carbamide, 380
 Iso-leucine, 139
 Iso-maltose, 86
 Isomerism, 86
 Isoprene, 204
 Isotopes, 9
 Iso-valerianic acid, 139

 Jaundice, 275

 Kallikrein, 414, 444
 Kelp, 38
 Kerasin, 159, 169, 170
 Keratin, 127
 Keto-acids, 305
 Ketogenesis, 319
 Ketogenic amino acids, 414
 Ketogenic principle, 438
 Keto-glutaric acid, 309
 Keto-hexose, 103
 Ketonæmia, 319
 Ketonuria, 319, 407
 Ketose test, 112
 Ketosis, 319
 Kryptoxanthine, 203
 Kynurenic acid, 310
 Kynurenin, 310

 Laccase, 35, 330
 Lactacidogen, 296
 Lactalbumin, 127
 Lactase, 88, 218
 Lactglobulin, 128
 Lactic acid, 142, 224, 272, 451
 Lactic dehydrogenase, 225, 32
 Lactone, 105
 Lactosazone, 115, 117
 Lactose, 87, 90, 301, 405
 Lactosuria, 302, 405
 Lævulinic acid, 103
 Lævulose. *See* Fructose, 82
 Lanolin, 167
 Lanosterol, 173
 Lard, 165
 Lauric acid, 318
 Lead, 28

- Lecithin**, 159, 167, 322
Legumelin, 127
Legumin, 128
Leucine, 139
Leucosin, 127
Levan, 96
Levosin, 96, 97
Liebig's law, 43
Lignocellulose, 97
Lignoceric acid, 170
Linoleic acid, 162, 251, 320
Linolenic acid, 162, 251, 320
Lipase, 216, 274
Lipides, 158-167
 absorption, 280
 metabolism, 316
Lipines, 158, 167
Lipochromes, 159, 200
Lipoids, 159
Liposis, 264
Lithium, 12
Lithocholic acid, 177
Liver oils, 165
Livetin, 129
Lorain syndrome, 440
Lumichrome, 206
Lumisterol, 180
Luteal hormones, 425
Luteinising factor (LH), 414, 430, 437
Luteosterone, 181, 429
Lycopene, 201
Lyochromes, 205, 255
Lyo-enzymes, 210
Lysine, 139
Lysolecithin, 169
- Magnesium**, 17, 214, 293, 236, 390, 451
Malic acid, 328
Malic dehydrogenase, 328
Maltase, 218
Maltoflavin, 205
Maltosazone, 115, 116
Maltose, 87, 90
Malt sugar. *See* Maltose.
Manganese, 35, 214
Mannan, 78
Mannitol, 98
Mannose, 82
Marine oils, 165
Melanin, 226, 309
Melezitose, 78, 86
Melibiose, 88
Melibiose, 86, 88
Melissyl alcohol, 162
Membrane, cell, 57
 Donnan effect, 58
 semi-permeable, 63
Menstrual cycle, 431
Mesobilin, 184
- Mesobilinogen**, 184
Metabolic principle, 414, 437
Metabolism, inborn errors, 315
Metaproteins, 132, 185
Methæm, 191, 193
Methæmoglobin, 190, 456
Methionine, 138, 310
Methylamine, 143, 310
Methyl cytosine, 130, 353
Methyl glyoxal, 224, 296
Methyl guanidine, 360
Methyl purines, 345
Methyl violet, 276
Methylene blue, 342
 test, 111
Milk, composition, 153
 fat, 165
 proteins, 129, 154
 sugar, 87, 301
Millon's test, 149, 400
Mineral nutrients, 236
Molisch's test, 109
Molybdenum, 32
Mononucleotides, 100, 347
Monosaccharides, 80, 81
Moore's test, 109
Mucic acid, 87, 105, 114, 117
Mucin, 130, 367
Mucoids, 130
Mucoitin sulphate, 98
Mucopolysaccharides, 96, 97
Mucoproteins, 130
Murexide test, 347
Muscarine, 363
Muscle, metabolism, 289-294
Mutarotation, 84
Mutase, 223, 227
Myalbumin, 127
Myoglobulin, 128
Myohæmatin, 195
Myricyl alcohol, 162
Myxœdema, 420
- Naphthalene**, 74
Naphthol test, carbohydrates, 112
 guanidines, 361
Naphthoresorcinol test, 112, 406
Nervone, 169
Nervonic acid, 170
Neuberg ester, 99, 292
Neurine, 363
Neurocrine, 364, 412
Neuro-humeral mechanisms, 364
Neutralisation curves, 52
Neutrality, 50, 51
Nickel, 41
Nicotinic acid, 258
Nicotinic amide, 258, 333
Night-blindness, 243
Ninhydrin test, 143, 148

- Nitrite, in saliva, 267
 Nitro-chromic test, 109, 166, 408
 Nitrogen, 28, 29
 Nitroglycerin, 163
 Nitroprusside test, 150, 334, 407
 Nonylic acid, 161
 Nucleic acid, 130, 137, 348
 Nucleinase, 279
 Nucleohistone, 126
 Nucleoprotein, 125, 129
 Nucleopurines, 344
 Nucleosidase, 279
 Nucleosides, 130, 347, 348
 Nucleotidase, 279
 Nucleotides, 130, 333
 Nyctalopia, 343
- Estradiol, 181, 428
 Estriol, 181
 Estrogenic factor, 437
 Estrogens, 428, 436
 Estrone, 180, 181, 436
 Oils, 159, 164
 Oleic acid, 161, 320
 Onium compounds, 361
 Organisers, 446
 Organism, composition, 42
 four-dimensional, 3
 Ornithine, 138, 385
 Ornithine cycle, 385-387
 Orosin, 128, 450
 Oryzenin, 126
 Osazones, 105, 115
 Osmotic pressure, 63
 Osteomalacia, 247
 Ostreasterol, 173
 Ovalbumin, 127, 143
 Ovarian hormones, 428
 Ovoflavin, 205
 Ovoglobulin, 128, 143
 Ovomucoid, 131
 Oxalates, 410
 Oxalic acid, 102
 Oxaloacetic acid, 304, 331, 333
 Oxidases, 224, 226, 328, 330
 classification, 337
 Oxidation-reduction potential, 339, 342
 Oxido-reductases, 224
 Oxygen, 32, 235
 Oxyhæmoglobin, 189, 195
 Oxyurines, 345
 Oxytocin, 438
- Palmitic acid, 161
 Pancreatic enzymes, 273
 Pancreatic hormones, 414, 424
 Pancreatic juice, 273
- Pancreotropic principle, 438
 Panthothenic acid, 258, 445
 Papain, 219
 Paracasein, 129
 Parahormones, 424
 Parasympathin, 365
 Parathyrin, 21, 422
 Parathyroid hormone, 422-424
 Parnas cycle, 293
 Pasteur effect, 288
 Pauly's test, 358
 Pectase, 97
 Pectin, 97
 Pellagra, 258
 Pellagra-preventing (p.p.) factor, 258, 240
 Pentosans, 78, 91
 Pentoses, 77, 81, 103, 282
 Pentosidase, 215
 Pentosuria, 81, 282, 315, 405, 409
 Pepsin, 219, 268, 269
 Peptidase, 215, 220
 Peptide theory, 145
 Peptides, 125, 133
 Peptones, 125, 133
 Periodic classification, 9
 Peroxidases, 89, 226, 328, 330
 Perspective formulæ, 85
 Pettenkofer's test, 277
 pH, notation, 51
 scale, 52
 Phenanthrene, 74, 172
 Phenols, urinary, 399
 Phenyl acetic acid, 319
 Phenylalanine, 137, 307, 308
 Phenylhydrazine, 105, 115
 Phenylpropionic acid, 319
 Phloridzin, 283, 301
 diabetes, 284
 Phosphagen, 31, 291, 367
 Phosphatase, 216, 278
 Phosphate, 31
 estimation, 395
 hexose, 99, 107, 290
 Phosphatides, 159, 167, 322
 Phosphatidic acid, 168
 Phosphocreatine, 216, 290
 Phosphoglyceric acid, 292, 107
 Phospholecithinase, 216
 Phospholipides, 159, 167, 322
 Phospholipines, 159
 Phosphoproteins, 125, 129
 Phosphoribose, 100
 Phosphorus, 30, 32
 isotope, 32
 Phosphorylation, 100
 Phosphotidates, 159
 Phrenosin, 169
 Phrenosinic acid, 170
 Phrynoderma, 242
 Phthioic acid, 160, 168

- Phylloerythrin, 184, 198, 275
 Phytochromes, 131
 Phytohormones, 436
 Phytol, 18, 101, 197, 251
 Phytosterols, 173
 Pilocarpine, 366, 442
 Pinnaglobulin, 35
 Pituitary factor, 300
 Pituitary hormones, 436-441
 Pituitrin, 439
 Placental hormone, 414, 429
 Plant autacoids, 436
 Polariscope, 79
 Polyases, 215
 Polyhexosides, 76, 90
 Polynucleotides, 347, 348
 Polyneuritis, 253
 Polyphenol oxidase, 330
 Polypeptide, 145
 Polysaccharidases, 215
 Polysaccharides, 76, 78, 90
 Porphin, 187
 Porphyrin, 187
 Porphyria, 187
 Porphyrins, 107, 198, 200
 Porphyrinuria, 188, 315, 409
 Potassium, 14, 236, 391, 398, 410
 P.p. factor, 258
 Pregnanediol, 181
 Progesterone, 181, 429
 Projection formulæ, 81
 Prolactin, 437
 Prolamins, 126
 Prolan, 430
 Proline, 140, 311
 Prolysine, 139
 Promoters, enzyme, 214
 Propionic acid, 137
 Prosthetic group, 129
 Protagon, 168
 Protamine insulinate, 23, 426
 Protamines, 125, 126
 Proteases, 215
 Protective foods, 265
 Protectors, enzyme, 214
 Protein, copper test, 144
 requirements, 238
 structure, 135, 157
 Proteinogenous amines, 356
 Proteins, 125-147, 237
 biological value, 238
 carbohydrate content, 134
 metabolism, 303-316
 Proteinuria, 401
 Proteoses, 125, 132
 Proteosuria, 401
 Proton, 48
 Protoporphyrin, 187, 193
 Provitamin A, 203, 241
 Provitamin D₂, 174
 Pseudo-muscarine, 363
 Ptomaine, 363
 Ptyalin, 217, 267
 Purines, 74, 344-353
 Putrescine, 359
 Pyramidon test, 403
 Pyran, 74, 85
 Pyranose, 84
 Pyridine, 74
 nucleotides, 333
 proteins, 331
 Pyrimidines, 74, 252, 344, 353
 nucleotides, 354
 Pyrocatechol, 400
 Pyrroles, 184
 Pyrrolidine, 140
 Pyruvic acid, 253, 299, 310

 Quinhydrone electrode, 50

 Radio-activity, 15, 32
 Radio-phosphorus, 32
 Radio-potassium, 15
 Raffinose, 78, 85, 89
 Raman effect, 67
 Redox potential, 340
 Reducing sugars, 111
 Refection, 258
 Rennin, 129, 254, 220, 269
 Renoflavin, 205
 Respiration, 289
 tissue, 325-342
 Respiratory carriers, 330
 enzymes, 328
 hamatins, 331
 quotient, 287
 Retinene, 243
 Reversion synthesis, 231
 Rhamnose, 77, 100
 Rhodopsin cycle, 243
 Rhodoxanthin, 201
 Riboflavin, 205, 206, 255, 332
 Ribose, 77, 80
 Ricinoleic acid, 162
 Rickets, 247
 Robison ester, 100, 332
 Rotation, specific, 108
 Rothera's test, 130, 150, 334, 407
 Rubidium, 17

 Saccharic acid, 104, 114
 Saccharidases, 215, 217
 Saccharide acids, 78, 98, 105
 alcohols, 78, 98, 104
 esters, 78, 99
 Saccharides, 76
 Saccharinic acids, 104
 Saccharose. *See* Sucrose, 89
 Sakaguchi's test, 361

- Saliva, 266
 Salmin, 126
 Saponification, 165
 Saponin, 179
 Sarcosine, 136, 367
 Scatole, 309, 310
 Schardinger enzyme, 227, 338
 Schiff's test, 347
 Scleroproteins, 125, 126
 Scurvy, 239, 261
 Scymnol, 177, 178
 Sea water, 60
 Secretin, 442
 Sedimentation rate, 69
 Seliwanoff's test, 112
 Sepia, 309
 Serine, 137, 307, 362
 Serum albumin, 127
 Serum globulin, 128
 Serum mucoid, 131
 Silicon, 27
 Silver, 17
 Sitosterol, 173, 174
 Soap, 166
 Soap wort, 179
 Sodium, 12-14, 59, 236, 390
 Sorbitol, 98
 Sørensen reaction, 142, 394
 Soret's band, 190
 Specific rotation, 108
 Specificity, enzyme, 212
 Spermaceti, 167
 Spermidine, 359
 Spermine, 359
 Sphingomyelin, 107, 159
 Sphingosinol, 168, 359
 Spongosterol, 173
 Stabilisers, colloid, 68
 Stachyose, 78, 86, 89
 Starch, 92, 96, 236
 hydrolysis, 117
 Stearic acid, 161, 320
 series, 160
 Stercobilin, 276
 Stereochrone, 3
 Stereo-isomerism, 79
 Steroids, 160, 177
 Steroid vitamins, 180
 Sterols, 173
 Stigmasterol, 173, 176
 Stilboestrol, 181, 433
 Strontium, 22
 Strophanthidin, 179
 Substrate, 214
 Succinic acid, 139, 313, 328
 dehydrogenase, 225, 323
 Succus entericus, 278
 Sucrase, 89, 218
 Sucrose, 89, 119, 278
 Sugar, 76-90
 blood, 283, 455
 Sugar estimation, 119-122, 455
 fermentation, 113, 297
 metabolism, 288-302
 Sullivan's test, 361
 Sulphaemoglobin, 189, 456
 Sulphate, 33, 396
 Sulphatase, 216
 urinary, 396
 Sulphatides, 159
 Sulpholipides, 33, 159
 Sulphur, 33
 Sulphur bacteria, 34
 Sulphydryl test, 334
 Suspensoid, 66
 Sympathin, 365
 Sympathomimetic amines, 356
 Synapsin, 366

 Tachysterol, 180
 Tallow, 165
 Tannase, 216
 Taraxanthin, 201
 Taurine, 177
 Taurocholic acid, 177, 277
 Tautomerism, 75
 Terpene, 160
 Testosterone, 182, 435
 Tetany, 424
 Tetra-pyrroles, 184, 187, 200
 Tetrasaccharides, 78, 89
 Tetrose, 77
 Theelin, 429
 Theelol, 429
 Thiamine, 252, 295
 Thiochrome, 252, 354
 Thiocyanate, 33, 267
 Thiols, 331, 334
 Thiomelanin, 34
 Thioneine, 314, 362
 Threonine, 138, 314
 Thymine, 354
 Thymol blue, 274
 Thymol test, 108, 401
 Thymo-nucleic acid, 130, 348
 Thymus histone, 126
 Thyroid hormone, 418
 Thyrotropic principle, 437
 Thyroxine, 38, 137, 308, 419
 Tiglic acid, 162
 Tin, 28
 Titanium, 28
 Toad poison, 179
 Tocopherol. *See* Vitamin E, 249
 Töpfer's reagent, 271
 Toxisterol, 180
 Transamination, 303
 Traumatic acid, 446
 Trehalase, 89
 Trehalose, 86, 89
 Trimethylamine, 374

- Trimethylamine oxide, 374
 Trisaccharidase, 218
 Trisaccharides, 78, 89
 Trommer's test, 110
 Trypsin, 219, 273
 Trypsinogen, 273
 Tryptamine, 352, 357
 Tryptophane, 138, 149, 309
 Tuberculo-stearic acid, 160
 Turacin, 16, 188
 Turanose, 86
 Turpentine test, 277, 404
 Tyramine, 143, 357
 Tyrosinase, 224, 226, 330
 Tyrosine, 137, 149, 307
 Tyrosinosis, 308, 315
- Ultra-centrifuge, 70
 Ultra-filtration, 69
 Unsaturation, 26
 Uracil, 354
 Uræmia, 388
 Urates, 440
 Urea, 268, 376-389, 396-398, 452
 Ureagenesis, 385-387
 Urease, 213, 222, 383
 Ureotelic, 257, 350
 Uric acid, 225, 344-347, 352, 398, 410
 Uricase, 225, 351
 Uricolytic index, 352
 Uricotelic, 257, 385
 Uridine, 354
 Urine, normal solutes, 390-401
 pathological solutes, 401-409
 sediments, 409
 Urobilin, 184, 186, 276, 392
 Urobilinogen, 184, 186, 276, 392
 Urochrome, 392
 Uroerythrin 393
 Uroflavin, 205
 Uronic acids, 104
 Uroporphyrin, 188, 393
 Uteroverdin, 184, 186
- Valerianic (valeric) acid, 138
 Valine, 139
 Vanadium, 28
 Van den Berg test, 277
 Van Slyke reaction, 142, 143
 Van t'Hoff's rule, 86
 Vaso-dilatin, 366
 Vaso-pressin, 366, 439
 Vegetable oils, 165
 Verdohæmochromogen, 199
 Vicine, 354
 Violerythrin, 204
 Virus proteins, 125,
 Visual purple, 243
- Vitamins, fat soluble, 241-251
 A, 203, 241-246
 A group, 203, 241-246
 D group, 179, 246-249
 E, 249-251
 F, 251, 320
 K, 251
 Vitamins, water-soluble, 240, 252-263
 B group, 252-259
 B₁, 33, 214, 228, 252-255, 336,
 354
 B₂, 206, 255-257, 354
 B₃, 257
 B₄, 257
 B₅, 257
 B₆, 257-258
 C, 98, 259-263, 336
 P, 240, 262
 P.p. factor, 214, 258, 333
 Vitellin, 129
- Warburg's respiration factor, 331
 Warburg's yellow enzyme, 332
 Water, 10, 45, 61, 235
 bound and free, 46
 heavy, 11
 Waxes, 159, 166
 World-volume, 3
 Wound hormone, 446
- Xanthine, 344, 225, 344
 Xanthine oxidase, 225, 351
 Xanthophyll, 200
 Xanthoprotein test, 149
 Xanthydrol test, 383
 Xerophthalmia, 242
 X-ray analysis, 145
 Xylans, 78, 80, 91, 97
 Xyloketose, 80, 405, 409
 Xylosazone, 115
 Xylose, 77, 80
- Yeast fermentation, 113, 297
 Yeast nucleic acid, 130, 348
 Yellow enzyme, 196, 205, 256, 331
- Zeaxanthin, 201
 Zein, 144, 150
 Zinc, 23, 228, 252, 420
 Zoosterols, 173
 Zwitter ions, 57, 136
 Zymase, 296, 298
 Zymoflavin, 205
 Zymoproteins, 125, 229, 331